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(54) Title: POLYVALENT IMMUNOGEN

(57) Abstract: The present invention relates, generally, to a polyvalent immunogen and, more particularly, to a method of inducing neutralizing antibodies against HIV and to a polyvalent immunogen suitable for use in such a method.

POLYVALENT IMMUNOGEN

This application claims priority from Provisional Application No. 60/471,327, filed May 19, 2003, the content of which is incorporated by reference.

TECHNICAL FIELD

The present invention relates, generally, to a polyvalent immunogen and, more particularly, to a method of inducing neutralizing antibodies against 5 HIV and to a polyvalent immunogen suitable for use in such a method.

BACKGROUND

Immunogenic peptides have been developed that elicit B and T cell responses to various strains of human immunodeficiency virus (HIV) (Palker et al, J. Immunol. 142:3612-3619 (1989), Haynes et al, Trans. Am. Assoc. 10 Physician 106:31-41 (1993), Haynes et al, J. Immunol. 151:1646-1653 (1993), Haynes et al, AID Res. Human Retroviruses 11:211-221 (1995)) (see also WO 97/14436). These peptides consist of two components, each derived from noncontiguous regions of the HIV gp120 envelope protein. One envelope component consists of 16 amino acid residues from the fourth constant (C4) 15 domain of HIV gp120, and includes a T-helper epitope (Cease et al, Proc. Natl. Acad. Sci. USA 84:4249-4253 (1987)). Linked to the carboxyl terminus of this gp120 C4 region peptide is a 23 amino acid segment from the third variable (V3) domain of gp120, that includes a B cell neutralizing antibody epitope for cell line-adapted HIV strains (Palker et al, J. Immunol. 142:3612- 20 3619 (1989), (Palker et al, Proc. Natl. Acad. Sci. USA 85:1932-1936 (1988), Rusche et al, Proc. Natl. Acad. Sci. USA 85:3198-3202)), a T-helper epitope (Palker et al, J. Immunol. 142:3612-3619 (1989)), and two cytotoxic T lymphopoitetic (CTL) epitopes (Clerici et al, J. Immunol. 146:2214-2219 (1991), Safrit et al, 6th NCVDG Meeting, Oct. 30 to Nov. 4, 1993)). In mice 25 and rhesus monkeys, these C4-V3 hybrid peptides have induced antibodies that bind to native gp120 and neutralize the particular cell line-adapted strain of HIV from which the V3 segment was derived, as well as induce T helper

cell proliferative responses and MHC Class I-restricted CTL responses that kill HIV or HIV protein expressing target cells (Palker et al, J. Immunol. 142:3612-3619 (1989), Haynes et al, AIDS Res. Human Retroviruses 11:211-221 (1995)). Recently, it was shown that this gp120 peptide design can induce 5 antibodies that neutralize primary HIV isolates and simian-human immunodeficiency viruses (SHIV) expressing primary HIV isolate envelopes (Liao et al, J. Virol. 74:254-263 (2000)). Moreover, in a challenge trial of this immunogen in rhesus monkeys, it was shown that C4-V3 peptides from the gp120 of the pathogenic SHIV 89.6P, induced neutralizing antibodies that 10 prevented the fall in CD4 counts after challenge with SHIV 89.6P (Letvin et al, J. Virol. 75:4165-4175 (2001)). Therefore, anti-V3 antibodies can protect primates against primary isolate SHIV-induced disease.

A prototype polyvalent HIV experimental immunogen comprised of the conserved C4 region of gp120 and the V3 regions of HIV isolates MN, 15 CANO(A), EV91 and RF has been constructed and has been found to be highly immunogenic in human clinical trials (Bartlett et al, AIDS 12:1291-1300 (1998), Graham et al, Abstract, AIDS Vaccine (2001)). Thus, understanding secondary and higher order structures of the components of this 20 polyvalent immunogen, as well as defining strategies to optimize gp120 immunogen antigenicity, is important to HIV vaccine design efforts. In addition, recent data suggest that the HIV V3 region may be involved in regulating gp120 interactions with HIV co-receptors, CXC chemokine receptor 4 (CXCR4) and chemokine receptor type 5 (CCR5) (Berger, AIDS Suppl. A:53-56 (1997)).

25 In previous studies, nuclear magnetic resonance (NMR) has been used to characterize conformations of the multivalent immunogen C4-V3 peptides in solution (de Lorimier et al, Biochemistry 33:2055-2062 (1994), Vu et al, Biochemistry 35:5158-5165 (1996), Vu et al, J. Virol. 73:746-750 (1999)). It has been found that the V3 segments of each of the four C4-V3 peptides 30 displayed evidence of preferred solution conformations, with some features

shared, and other features differing among the four peptides. The C4 segment, which is of identical sequence in all the peptides, showed in each case a tendency to adopt nascent helical conformations (de Lorimier et al, Biochemistry 33:2055-2062 (1994), Vu et al, Biochemistry 35:5158-5165 5 (1996), Vu et al, J. Virol. 73:746-750 (1999)).

The C4 sequence as a peptide does not elicit antibodies that bind native gp120 (Palker et al, J. Immunol. 142:3612-3619 (1989), Haynes et al, J. Immunol. 151:1646-1653 (1993), Ho et al, J. Virol. 61:2024-2028 (1987), Robey et al, J. Biol. Chem. 270:23918-23921 (1995)). This led to the 10 speculation that the nascent helical conformations exhibited by the C4 segment might reflect a conformation not native to HIV gp120. Amino-acid sequence homology between the gp120 C4 region and a human IgA CH1 domain has been noted (Maddon et al, Cell 47:333-348 (1986)). By comparison to the structure of mouse IgA (Segal et al, Proc. Natl. Acad. Sci. 15 USA 71:4298-4302 (1974)), the C4-homologous region of IgA has a β strand secondary structure (de Lorimier et al, Biochemistry 33:2055-2062 (1994)). Therefore, while the C4 gp120 peptide in solution adopts nascent helical conformations, the native structure of this gp120 C4 region may be quite different (ie, in the context of gp 120 have a β strand secondary structure).

20 The present invention results, at least in part, from the results of a study with a three-fold purpose. First, C4-V3HIVRF peptides with amino acid substitutions designed to minimize C4 α -helical peptide conformation and promote β strand C4 secondary structures were constructed in order to induce anti-native gp120 antibodies with the modified C4 peptide. Second, tests were 25 made to determine if any of these mutated C4-V3RF peptides would enhance gp120 V3 region peptide immunogenicity, and therefore augment anti-HIVRF gp120 V3 loop antibody responses. Finally, the solution conformers of each peptide studied immunologically were also solved using NMR to correlate peptide conformers with peptide immunogenicity.

SUMMARY OF THE INVENTION

The present invention relates to a method of inducing neutralizing antibodies against HIV and to peptides, and DNA sequences encoding same, that are suitable for use in such a method.

In one embodiment, the invention relates to a composition comprising a multiplicity of immunogenic peptides comprising a first and a second component, the first component comprising a T-helper epitope, the second component comprising residues of the V3 domain of gp120 and including a B cell neutralizing antibody epitope. The first component can be a human immunodeficiency virus (HIV) T helper epitope. The first component can comprise residues of the C4 domain of HIV gp120, for example, at least 16 contiguous residues of the C4 domain of HIV gp120 (e.g., residues 421 to 436 of the C4 domain of HIV gp120). The first component can comprise residues of HIV p24 gag (e.g., GTH1 (residues 262-278 of HIV gag)). The first component can be a non-HIV T helper epitope. The second component can comprise at least 23 contiguous residues of the V3 domain of HIV gp120 (e.g., residues 297 to 322 of the V3 domain of HIV gp120). The first component can comprise at least 16 contiguous residues of the C4 domain of HIV gp120 and the second component can comprise at least 23 contiguous residues of the V3 domain of HIV gp120 (e.g., residues 421 to 436 of the C4 domain of HIV gp120 and residues 297 to 322 of the V3 domain of HIV gp120). The second component can be linked C terminal to the first component. The first component can be linked to the second component via a linker. The composition can comprise at least 5 immunogenic peptides (e.g., C4-V3 36.29, C4-V3 34.29, C4-V3 62.19, C4-V3 74.17 and C4-V3 162.7 from Table 7). The composition can comprise at least 10, or at least 25 immunogenic peptides. The composition can further comprise a carrier and/or an adjuvant. The first component can comprise the sequence YKRWIILGLNKIVRM. The second components can be selected so as to be

representative of higher order structural motifs present in a population, which motifs mediate V3 functions in the course of envelope mediated HIV interaction with host cells. The composition can comprise about 25-30 immunogenic peptides the second components of which are selected so as to 5 be representative of infected individuals within a subtype. At least one of the first components can comprise the sequence KQIINMWQVVGKAMYA. This aspect of the invention further relates to a method of inducing the production of neutralizing antibodies in a patient comprising administering to the patient an amount of the above composition sufficient to effect the 10 production. In another aspect of this embodiment, the invention relates to a formulation comprising at least one nucleic acid sequence encoding the above composition and to a method of inducing the production of neutralizing antibodies in a patient comprising administering to the patient an amount of the formulation sufficient to effect the production..

15 In another embodiment, the invention relates to a composition comprising at least one peptide from Table 6 or Table 7 and a carrier. This composition can further comprise an adjuvant. In another embodiment, the invention relates to a nucleic acid encoding a peptide in Table 6 or Table 7.

In a further embodiment, the invention relates to an isolated 20 polypeptide comprising a V3 sequence shown in Table 10, 11 or 12 (e.g., polypeptide 62.19) and to an isolated nucleic acid sequence encoding at least one such polypeptide. The invention further relates to a vector comprising such a nucleic acid. Additionally the invention relates to a method of inducing the production of neutralizing antibodies in a mammal comprising 25 administering to the mammal an amount of such a polypeptide or nucleic acid sequence sufficient to effect the induction (e.g., the V3 sequence can be administered in a DNA prime with the V3 sequence in a gp140 or gp160 boost (or a gp120, gp140 or gp160 replication vector boost) (a replicating vector comprising the V3 sequence in envelope can be administered as prime and

boost). The invention further relates to a composition comprising such a polypeptide or nucleic acid sequence and a carrier.

Other embodiment, objects and advantages of the present invention will be clear from the description that follows.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Summary of antibody binding titers to immunizing peptide after 2 or 3 boosts of 3 mice in each group with immunizing peptide. There was a slight enhancement of levels of antibody induced by the E9G variant after 2 but not 3 boosts, while the E9V variant significantly boosted antibody levels compared to the C4-V3RF(A) peptide after 2 and 3 boosts.
10 Antibody to the K12E variant induced by the K12E peptide was significantly lower than C4-V3RF(A) induced antibody levels after both 2 and 3 boosts.

Figures 2A, 2B, 2C, 2D: NMR spectra of the four C4-V3RF variant peptides.

Figures 3A, 3B, 3C, 3D: C4_{E9V}-V389.6 peptides bound better to human PB lymphocytes and monocytes than did the C4-V3 89.6 peptides. Similar data were obtained with the C4-V3 89.6P and C4-E9V-89.6P peptides. Sequence of the C4-V389.6 peptide form HIV89.6 isolate was:
KQIINMWQEVGKAMYA-TRPNNNTRRLSIGPGRAFYARR; the sequence of the C4_{E9V}-V389.6 peptide was: KQIINMWQVVGKAMYA-
20 TRPNNNTRRLSIGPGRAFYARR; the sequence of the C4-V389.6P peptide was: KQIINMWQEVGKAMYA-TRPNNNTRERLSIGPGRAFYARR; the sequence of the C4E9V-V389.6P peptide was: KQIINMWQVVGKAMYA-TRPNNNTRERLSIGPGRAFYARR.

Figure 4: Neutralization of BAL in PBMC.

Figure 5: Neutralization of HIV primary isolates by sera from guinea pig (GP) 469 immunized with the C4-V3 peptide 62.19. The isolates tested are listed on the right side. The grey and white areas indicate no neutralization. The red boxes indicate >50% neutralization. The titers are
5 1:10, 1:30, 1:90 and 1:270 going across in each column.

Figure 6: C4-V3 sequences tested.

Figure 7: Strategy for design of HIV gp120 immunogens with higher order structures.

DETAILED DESCRIPTION OF THE INVENTION

10 The present invention relates, at least in part, to a composition comprising a multiplicity of immunogenic hybrid peptides, each comprising two components. One component includes a T-helper epitope and can comprise residues from the C4 domain of HIV gp120. The second component comprises residues from the V3 domain of gp120 and includes a B cell
15 neutralizing antibody epitope.

Advantageously, the first component comprises about 16 contiguous residues from the C4 domain (about residues 421 to 436) and the second component comprises about 23-25 contiguous residues from the V3 domain (about residues 297 to 322). The components can, however, be longer, and
20 can comprise, for example, the entirety of the cysteine to cysteine V3 loop region, or be shorter. Preferably, the V3 component is linked C terminal to the C4 component peptide. The hybrid peptides can include additional sequences (e.g., linkers (e.g., cysteine, serine or lysine linkers) between the C4 and V3 components). The composition can, for example, comprise 5 to 10 hybrid
25 peptides, 10 to 15 hybrid peptides or 25 to 30 hybrid peptides. The number of hybrid peptides used will depend, at least in part, on the target population.

Preferred first components comprising residues from the C4 domain are shown in the Tables that follow (see particularly Tables 6 and 7). Other T helper determinants from HIV or from non-HIV proteins can also be used. For example, a further T helper epitope suitable for use in the invention is 5 from HIV gag (e.g., residues 262-278). One such sequence, designated GTH1, is YKRWIILGLNKIVRMYS (from HIV p24 gag). Variants of this sequence can also be used. Alternatively, or in addition, a carbohydrate such as the outer membrane protein of pneumococcus, or another carbohydrate or protein with immunogenic, T helper activity can be used.

10 The V3 components of the hybrid peptides present in the instant composition are selected so as to be representative of higher order structural motifs present in a population, which motifs mediate V3 functions in the course of envelope mediated HIV interaction with host cells. The Los Alamos National Laboratories Human Retroviruses and AIDS Database (Human Retroviruses and AIDS, 2000, Published by the Theoretical Biology and Biophysics G T-10, Mail Stop K710, LANL, Los Alamos, NM) presently contains over 14,000 HIV V3 envelope sequences, showing the extraordinary diversity the virus has obtained since originating in man in Africa approximately 50 years ago. For example, among 432 HIV-1 V3 sequences 15 derived from individuals infected with subtype C (designated "Clade C") in Africa currently available in the HIV database, 176 distinct variants of a 23 amino acid stretch at the tip of the V3 loop have been found. Similarly, among 6870 B subtype (designated "Clade B") V3 sequences from the US, 20 1514 unique forms have been found.

25 A method has been developed to organize short antigenic domains by protein similarity scores using maximum-linkage clustering. This method enables the visualization of the clustering patterns as a dendrogram, and the splitting patterns in the dendrogram can be used to define clusters of related sequences (Korber et al, J. Virol. 68:6730-6744 (1994)). The method allows 30 the use of several different amino acid similarity scoring schemes available in

the literature, preferred is the amino acid substitution matrix developed by Henikoff and Henikoff (see Advances in Protein Chemistry 54:73-97 (2000) and Proteins: Structure, Function and Genetics 17:49-61 (1993)), designed to give substitutions that are well tolerated in conserved protein structural elements a high score, and a low score to those that are not. Typically excluded from consideration very rare, highly divergent peptides, and favored are peptides found in many individuals within the population. In a selected set of sequences , most of the unique forms are within one or two amino acids from a least one other of the peptides chosen. This method has been applied to clustering the large number of variants of the antigenic tip of the V3 domain within Clade B and Clade C into groups (about 25) that are likely to be cross-reactive within the group. Based on these clustering patterns, variants (e.g., about 25-30) are selected that are representative or "central" to each group, for testing for antigenicity. The HIV Clade B and Clade C gp120 envelope V3 sequences have been analyzed, as described above, for groups of V3 sequences predicted to have structural similarities. Twenty five Clade C and 30 Clade B groups have been defined, and chosen out of each group is a common, or the most common, sequence as a representative of that group. The selected V3 sequences have been included in a C4-V3 design thereby providing a 25 peptide Clade C immunogen, and a 30 peptide Clade B immunogen (see Tables 6 and 7).

Table 6

C4-V3 design of Clade C V3 sequences

- C4-V3-C1 **KQIINMWQVVGKAMYA**-trpnnntrksirigpGqtfyatg
- C4-V3-C2 **KQIINMWQVVGKAMYA**-trpnnntrksirigpGqtfyaRg
- 5 C4-V3-C3 **KQIINMWQVVGKAMYA**-trpnnntrksirigpGqtfyaAg
- C4-V3-C4 **KQIINMWQVVGKAMYA**-IrpnnntrksVrigpGqtfyatg
- C4-V3-C5 **KQIINMWQVVGKAMYA**-trpnnntrksirigpGqtfFatg
- C4-V3-C6 **KQIINMWQVVGKAMYA**-trpnnntrksirigpGqtfyatN
- C4-V3-C7 **KQIINMWQVVGKAMYA**-trpnnntrEsirigpGqtfyatg
- 10 C4-V3-C8 **KQIINMWQVVGKAMYA**-trpnnntrRsirigpGqAfyatg
- C4-V3-C9 **KQIINMWQVVGKAMYA**-trpnnntrkGirigpGqtfyatg
- C4-V3-C10 **KQIINMWQVVGKAMYA**-trpSnntrksirigpGqAfyatg
- C4-V3-C11 **KQIINMWQVVGKAMYA**-trpSnntrksirigpGqtfyatN
- C4-V3-C12 **KQIINMWQVVGKAMYA**-trpSnntrEsirigpGqtfyatg
- 15 C4-V3-C13 **KQIINMWQVVGKAMYA**-trpnnntrksMrigpGqtfyatg
- C4-V3-C14 **KQIINMWQVVGKAMYA**-trpGnntrksMrigpGqtfyatg
- C4-V3-C15 **KQIINMWQVVGKAMYA**-trpGnntrksirigpGqtLyatg
- C4-V3-C16 **KQIINMWQVVGKAMYA**-VrpnnntrksVrigpGqtSyatg
- C4-V3-C17 **KQIINMWQVVGKAMYA**-trpGnntrRsirigpGqtfyatg
- 20 C4-V3-C18 **KQIINMWQVVGKAMYA**-IrpGnntrksVrigpGqtfyatg
- C4-V3-C19 **KQIINMWQVVGKAMYA**-trpnnntrksirigpGqAfyatN
- C4-V3-C20 **KQIINMWQVVGKAMYA**-trpnnntrQsirigpGqAfyatK
- C4-V3-C21 **KQIINMWQVVGKAMYA**-trpGnntrksirigpGqAfFatg
- C4-V3-C22 **KQIINMWQVVGKAMYA**-trpGnntrksVrigpGqAfyatN
- 25 C4-V3-C23 **KQIINMWQVVGKAMYA**-trpnnntrkGiHigpGqAfyaAg
- C4-V3-C24 **KQIINMWQVVGKAMYA**-trpnnntrkGiGigpGqtfFatE
- C4-V3-C25 **KQIINMWQVVGKAMYA**-trpGnntrEsiGigpGqAfyatg

Table 7

C4-V3 peptides Clade B

C4-V3-396.2 KQIINMWQVVGKAMYA-RPNNNTRNIHIGLGRFYAT-*
 C4-V3-170.6 KQIINMWQVVGKAMYA-RPNNNTRRSVRIGPGGAMFRTG*
 5 C4-V3-82.15 KQIINMWQVVGKAMYA-RPNNNTRRSIPIGPGRFYTTG*
 C4-V3-144.8 KQIINMWQVVGKAMYA-RPDNNTRVKIPIGPGRSSFYTT-*
 C4-V3-23.38 KQIINMWQVVGKAMYA-RPIKIERKRIPGLGKAFYTTK*
 C4-V3-365.2 KQIINMWQVVGKAMYA-RPSNNTRKGIIHLGPGRAYATE*
 C4-V3-513.2 KQIINMWQVVGKAMYA-RPSNNTRKGIIHMGPGKAIYTTD*
 10 C4-V3-1448.1 KQIINMWQVVGKAMYA-RPGNTTRRGIPIGPGRAFFTTG*
 C4-V3-69.18 KQIINMWQVVGKAMYA-RPNNNTRKSIRIGPGRAYATD*
 C4-V3-146.8 KQIINMWQVVGKAMYA-RPGNNTRRRISIGPGRFAVATK*
 C4-V3-113.1 KQIINMWQVVGKAMYA-RPNNNTRRSIHLGMGRALYATG-*
 C4-V3-51.23 KQIINMWQVVGKAMYA-RPSNNTRRSIHMLGRAFYTTG-*
 15 C4-V3-72.18 KQIINMWQVVGKAMYA-RPNNNTRKGINIGPGRFYATG-*
 C4-V3-36.29 KQIINMWQVVGKAMYA-RPNNNTRKGIIHGPGRFFATG-*
 C4-V3-70.18 KQIINMWQVVGKAMYA-RPNNNTRKRIRIGHIGPGRFYATG*
 C4-V3-89.14 KQIINMWQVVGKAMYA-RPSINKRRHIHIGPGRFYAT-*
 C4-V3-163.7 KQIINMWQVVGKAMYA-RLYNYRRKGIIHGPGRAYATG*
 20 C4-V3-57.20 KQIINMWQVVGKAMYA-RPNRHTGKSIRMLGRAWHTTR-*
 C4-V3-11.85 KQIINMWQVVGKAMYA-RPNNNTRKSINIGPGRFYTTG---*
 C4-V3-34.29 KQIINMWQVVGKAMYA-RPNNNTRKSIQIGPGRFYTTG---*
 C4-V3-1.481 KQIINMWQVVGKAMYA-RPNNNTRKSIHIGPGRFYTTG---*
 C4-V3-85.15 KQIINMWQVVGKAMYA-RPNNNTRKSIHIAPGRFYTTG---*
 25 C4-V3-62.19 KQIINMWQVVGKAMYA-RPNNNTRKSIHIGPGRFYATE-----*
 C4-V3-125.9 KQIINMWQVVGKAMYA-RPNNNTRRRISMGPGRVLYTTG*
 C4-V3-35.29 KQIINMWQVVGKAMYA-RPNNNTRKRISLGPGRVYYTTG*
 C4-V3-74.17 KQIINMWQVVGKAMYA-RPNNNTRKRMTLGPGRKFYTTG*
 C4-V3-46.26 KQIINMWQVVGKAMYA-RPDNTIKQRJIHIGPGRPFYTT-*
 30 C4-V3-122.9 KQIINMWQVVGKAMYA-RPNYNETKRJRIHRGYGRSFVTVR*
 C4-V3-162.7 KQIINMWQVVGKAMYA-RPGNNTRGSIHLHPGRKFYYSR*
 C4-V3-3.323 KQIINMWQVVGKAMYA-RPNNNTRKSINMGPGRFYTTG

While the above is offered by way of example, it will be appreciated that the same analyses can be performed for HIV Clades A, D, E, F, G, H, M, N, O, etc, to design V3 immunogens that react with HIV primary isolates from these Clades.

5 In addition to the sequences described in Tables 6 and 7, a substitution has been made in the C4 sequence at position 9 from E to V to enhance the binding of the C4 region to human immune cell membranes, and to increase immunogenicity (see Example that follows). Substituting V for E at position 9 of C4 results in the C4-E9V-V3RF(A) peptide inducing 2-3 logs higher anti-
10 gp 120 V3 region antibody levels compared with the original C4-V3RFA(A) peptide. The effect of the E9V substitution is not species specific. While not wishing to be bound by theory, the data may indicate that the ability of the E9V variant peptide to enhance B cell antibody production is not MHC specific but rather it relates in some manner to non-MHC specific factors, such
15 as the ability of the peptides to bind to the lipid bilayer of immune cells. The data presented in Figure 3 demonstrate the ability of C4_{E9V}-V389.6 peptides to bind to human PB lymphocytes and monocytes. The ability of the C4 and C4E9V "T helper" determinants to facilitate immunogenicity of the V3 region may be due to the ability of helical amphipathic structures to interact with
20 lipid bilayers in a non-MHC related manner and promote peptide internalization. The invention encompasses the use of C4 sequences in addition to those described above.

In addition to the composition described above, the invention encompasses each of the hybrid peptides disclosed as well as each of the components (C4 and V3), alone or in covalent or non-covalent association
25 with other sequences, as well as nucleic acid sequences encoding any and all such peptides. The invention provides an HIV immunogen that can induce broadly reactive neutralizing antibodies against HIV of multiple quasispecies, and across clades. With reference to Example 3, the "dual D" HIV isolate,
30 neutralized by serum from GP 469 immunized with peptide 62.19 to a titer of

1:30, is a Clade A/G recombinant HIV isolate. This demonstrates that this peptide (62.19), for example, can induce antibodies against a non-B HIV isolate. The 62.19 and other V3 sequences in Figure 6 and Tables 10, 11 and 12 can be expressed either alone or, for example, as a C4-V3 sequence, as in 5 Figure 6. It will be appreciated that the same analysis described in Example 3 can be performed for any of HIV Clades A, D, E, F, G, H, M, N, O, etc, to identify V3 immunogens that react with HIV primary isolates from one or more of these Clades.

The peptide immunogens of the invention can be chemically 10 synthesized and purified using methods which are well known to the ordinarily skilled artisan. (See, for example, the Example that follows.) The composition can comprise the peptides linked end to end or can comprise a mixture of individual peptides. The peptide immunogens can also be synthesized by well-known recombinant DNA techniques. Recombinant 15 synthesis may be preferred when the peptides are covalently linked. Nucleic acids encoding the peptides of the invention can be used as components of, for example, a DNA vaccine wherein the peptide encoding sequence(s) is/are administered as naked DNA or, for example, a minigene encoding the peptides can be present in a viral vector. The encoding sequence(s) can be present, for 20 example, in a replicating or non-replicating adenoviral vector, an adeno-associated virus vector, an attenuated mycobacterium tuberculosis vector, a Bacillus Calmette Guerin (BCG) vector, a vaccinia or Modified Vaccinia Ankara (MVA) vector, another pox virus vector, recombinant polio and other enteric virus vector, Salmonella species bacterial vector, Shigella species 25 bacterial vector, Venezuelan Equine Encephalitis Virus (VEE) vector, a Semliki Forest Virus vector, or a Tobacco Mosaic Virus vector. The encoding sequence(s), can also be expressed as a DNA plasmid with, for example, an active promoter such as a CMV promoter. Other live vectors can also be used to express the sequences of the invention. Expression of the immunogenic 30 peptides of the invention can be induced in a patient's own cells, by

introduction into those cells of nucleic acids that encode the peptides, preferably using codons and promoters that optimize expression in human cells. Examples of methods of making and using DNA vaccines are disclosed in U.S. Pat. Nos. 5,580,859, 5,589,466, and 5,703,055.

5 The composition of the invention comprises an immunologically effective amount of the peptide immunogens of this invention, or nucleic acid sequence(s) encoding same, in a pharmaceutically acceptable delivery system. The compositions can be used for prevention and/or treatment of immunodeficiency virus infection. The compositions of the invention can be
10 formulated using adjuvants, emulsifiers, pharmaceutically-acceptable carriers or other ingredients routinely provided in vaccine compositions. Optimum formulations can be readily designed by one of ordinary skill in the art and can include formulations for immediate release and/or for sustained release, and for induction of systemic immunity and/or induction of localized mucosal
15 immunity (e.g., the formulation can be designed for intranasal administration). The present compositions can be administered by any convenient route including subcutaneous, intranasal, oral, intramuscular, or other parenteral or enteral route. The immunogens can be administered as a single dose or multiple doses. Optimum immunization schedules can be readily determined
20 by the ordinarily skilled artisan and can vary with the patient, the composition and the effect sought. By way of example, it is noted that approximately 50 μ g-100 μ g of each hybrid peptide can be administered, for example, intramuscularly (e.g. 3x).

25 The invention contemplates the direct use of both the peptides of the invention and/or nucleic acids encoding same and/or the peptides expressed as minigenes in the vectors indicated above. For example, a minigene encoding the peptides can be used as a prime and/or boost. Importantly, it has been recently shown that recombinant gp120 is not efficacious as a vaccine for HIV in phase III trials (Elias, P., Durham Morning Herald, Feb. 25, 2003; VaxGen
30 News Conference, February 24, 2003). Thus, it would be advantageous to

express, for example, the 62.19 V3 loop and/or other V3 loops in Table 11 or 12 in the context of gp120 molecules or gp160 or gp140 molecules, either as expressed soluble recombinant proteins, or expressed in the context of one of the vectors described above. This strategy takes advantage of the ability to 5 express native V3 conformations within a whole gp120 or gp140 or gp160 HIV envelope protein.

One of the preferred gp120, gp140 or gp160 envelopes that, for example, 62.19 V3 loops can be expressed with is that of consensus or ancestral HIV envelope artificial sequences (Gaaschen et al, Science 10 296:2354-2360 (2002)). Although artificial and computer designed, one such sequence (the consensus of consensus envelope) gp120 (con 6) has been shown to bind soluble CD4 and anti-gp120 mabs A32, 1b12, 2G12. After binding mab A32 or soluble CD4, the con 6 gp120 binds the CCR5 binding site mab 176 – indicating a "native" gp120 conformation.

15 Thus, the entire V3 loops from the Los Alamos Database from the sequences of one or more of the peptides in Table 11 or 12 can be expressed in the consensus (con 6) or other consensus or ancestral gp120, gp140, or gp160 envelope protein, or expressed in a native gp120, gp140, or gp160, such as HIV BAL or HIV JRFL, and used as an immunogen as a recombinant 20 envelope protein, or used as an immunogen expressed in one of the vectors above.

The V3 peptides or recombinant proteins can be used as primes or boosts with the V3 peptides or recombinant gp120s, gp140s or gp160s expressed in the above vectors used as primes or boosts.

25 A preferred immunogen is the consensus 6 gp120 expressing the full-length 62.19 V3 loop, expressed as a DNA plasmid as a primary immunization, followed by adenovirus expressing the Con 6 envelope expressing the 62.19 V3 sequence from the Los Alamos Database as a booster immunization.

In addition to the polypeptides described above and in the Examples that follow, the invention further relates to modified forms thereof (and nucleic acid sequences encoding same) wherein negatively charged amino acids are added/substituted on the right hand side of the loop and positively charged amino acids are added/substituted on the left hand side. Such additions/substitutions can serve to stabilize the "hairpin" as a result of hydrogen bonding between the oppositely charged amino acids. Conversely, negatively charged amino acids can be added/substituted on the left hand side of the loop and positively charged on the right. Examples of such sequences are as follows (in the "B" and "C" type polypeptides shown below, it will be appreciated that the D and the E can be reversed as well at the ends of the peptides – further, for example, DDD can be present at the right hand side of the loop, as can EEE):

Table 5

15 For 62.19

62.19A	C4-T R P N N N T R K S I H I G P G R A F Y A T E D
62.19B	C4-T R P N N N T R K S I H I G P G R A F Y A T E D E
62.19C	C4-T R P N N N R R K S I H I G P G R A F Y A T E D E
20 62.19D	T R P N N N T R K S I H I G P G R A F Y A T E D
62.19E	T R P N N N T R K S I H I G P G R A F Y A T E D E
62.19F	T R P N N N R R K S I H I G P G R A F Y A T E D E
62.19G	C4-T R P N N N R R K S I H I G P G R A F Y A T E E E
62.19H	T R P N N N R R K S I H I G P G R A F Y A T E E E

25

For 1.481

1.481A	C4-R P N N N T R K S I H I G P G R A F Y T T G D
1.481B	C4-R P N N N T R K S I H I G P G R A F Y T T G D E
30 1.481C	C4-R P N N N R R K S I H I G P G R A F Y T T G D E
1.481D	C4-R P N N N R R K S I H I G P G R A F Y T T G E E
1.481E	R P N N N T R K S I H I G P G R A F Y T T G D
1.481F	R P N N N T R K S I H I G P G R A F Y T T G D E
1.481G	R P N N N R R K S I H I G P G R A F Y T T G D E
35 1.481H	R P N N N R R K S I H I G P G R A F Y T T G E E

For 11.85

	11.85A	C4-R P N N N T R K S I N I G P G R A F Y T T G D
	11.85B	C4-R P N N N T R K S I N I G P G R A F Y T T G D E
5	11.85C	C4-R P N N R T R K S I N I G P G R A F Y T T G D E
	11.85D	C4-R P N N R T R K S I N I G P G R A F Y T T G E E
	11.85E	R P N N N T R K S I N I G P G R A F Y T T G D
	11.85F	R P N N N T R K S I N I G P G R A F Y T T G D E
	11.85G	R P N N R T R K S I N I G P G R A F Y T T G D E
10	11.85H	R P N N R T R K S I N I G P G R A F Y T T G E E

For 513.2 (R P S N N T R K G I H M G P G K A I Y T T D)

	513.2A	C4-R P S N N T R K G I H M G P G K A I Y T T D D
15	513.2B	C4-R P S N N T R K G I H M G P G K A I Y T T D D E
	513.2C	C4-R P S N N R R K G I H M G P G K A I Y T T D E
	513.2D	C4-R P S N N N R R K G I H M G P G K A I Y T T D E E
	513.2E	R P S N N T R K G I H M G P G K A I Y T T D D
	513.2F	R P S N N T R K G I H M G P G K A I Y T T D D E
20	513.2G	R P S N N R R K G I H M G P G K A I Y T T D E
	513.2H	R P S N N N R R K G I H M G P G K A I Y T T D E E

For 74.17

	74.17A	C4-R P N N N T R K R M T L G P G K V F Y T T G D
	74.17B	C4-R P N N N T R K R M T L G P G K V F Y T T G D E
	74.17C	C4-R P N N N R R K R M T L G P G K V F Y T T G D E
	74.17D	C4-R P N N N R R K R M T L G P G K V F Y T T G E E
	74.17E	R P N N N T R K R M T L G P G K V F Y T T G D
30	74.17F	R P N N N T R K R M T L G P G K V F Y T T G D E
	74.17G	R P N N N R R K R M T L G P G K V F Y T T G D E
	74.17H	R P N N N R R K R M T L G P G K V F Y T T G E E

For 36.29

35	36.29A	C4-R P N N N T R K G I H I G P G R T F F A T G D
	36.29B	C4-R P N N N T R K G I H I G P G R T F F A T G D E
	36.29C	C4-R P N N N R R K G I H I G P G R T F F A T G D E
	36.29B	C4-R P N N N R R K G I H I G P G R T F F A T G E E
40	36.29E	R P N N N T R K G I H I G P G R T F F A T G D
	36.29F	R P N N N T R K G I H I G P G R T F F A T G D E
	36.29G	R P N N N R R K G I H I G P G R T F F A T G D E
	36.29H	R P N N N R R K G I H I G P G R T F F A T G E E

For 34.29

5 34.29A C4-R P N N N T R K S I Q I G P G R A F Y T T G D
 34.29B C4-R P N N N T R K S I Q I G P G R A F Y T T G D E
 34.29C C4-R P N N N R R K S I Q I G P G R A F Y T T G D E
 34.29D C4-R P N N N T R R K S I Q I G P G R A F Y T T G E E
 34.29E R P N N N T R K S I Q I G P G R A F Y T T G D
 34.29F R P N N N T R K S I Q I G P G R A F Y T T G D E
 34.29G R P N N N R R K S I Q I G P G R A F Y T T G D E
 34.29H R P N N N T R R K S I Q I G P G R A F Y T T G E E

10 In the context of the modified forms of 62.19 depicted above, the invention further includes peptides wherein in the R in ...GPGR... (designated with an asterisk) can be substituted with any one of Q, G, K, S, A, L or H.

15 It will be appreciated from a reading of this disclosure that the foregoing, like others described herein, can be expressed, for example, in gp120, gp140 and that the vectors described above are equally applicable here.

15 Certain aspects of the invention can be described in greater detail in non-limiting Example that follows. (See also Figure 7.)

EXAMPLE 1Experimental Details*Peptide design, synthesis and purification.*

20 Peptides were designed, as shown in Table 1. It was hypothesized that alteration of the C4 sequence to reduce its helical conformational tendency in peptides might cause enrichment of solution conformers resembling a β strand conformation. This in turn might cause C4 to be immunogenic for antibodies recognizing the native conformation of the C4 (part of the CD4 binding site) region of gp120. The present work describes tests of this hypothesis in chimeric peptide C4-V3 RF, which has a V3 segment from gp120 of HIV strain RF, and three sequence variants wherein single amino-acid replacements have been introduced at position 9 in the C4 segment, Glu (E) to Gly (G), Glu (E) to Val (V), and at position 12, Lys (K) to Glu (E) (Table 1). These 25 replacements were made in part to disrupt possible stabilization of helical conformations due to side-chain ($i, i+3$) charge interaction between E9 and K12 (Scholtz et al, Biochemistry 32:9668-9676 (1993)). In addition, the 30

substitution in C4_{E9G}-V3RF(A) was expected to disfavor helix formation by introducing greater main-chain flexibility (Chakrabartty et al, Adv. Protein Chem. 46:141-176 (1995)). Furthermore the substitution in C4_{E9G}-V3RF(A) introduced two adjacent valine residues which has been hypothesized to favor 5 extended conformations. Thus, the parent peptide, C4-V3RF(A) (Haynes et al, AIDS Res. Human Retroviruses 11:211-221 (1995)) contained 16 N-terminal residues from the C4 domain of gp120_{IIIB} and 23 C-terminal residues from the V3 domain of gp120 of HIVRF.

TABLE 1
Peptides Used in This Study

Peptide	Sequence	C4	16	17	39	V3
C4-V3RF(A)	KQIINMWQEVGKAMYA				TRPNNNTRKSITKGPGRVIVYATG	
C4 _{E9G} -V3RF(A)	KQIINMWQGVGKAMYA				TRPNNNTRKSITKGPGRVIVYATG	
C4 _{E9V} -V3RF(A)	KQIINMWQVVGKAMYA				TRPNNNTRKSITKGPGRVIVYATG	
C4 _{K12E} -V3RF(A)	KQIINMWQEVGAEAMYA				TRPNNNTRKSITKGPGRVIVYATG	

Peptides were synthesized by fluorenylmethoxycarbonyl chemistry on an ABI 431A peptide synthesizer (Applied Biosystems, Inc., Foster City, CA), then purified by reverse-phase high performance liquid chromatography. The purity and identity of the product were confirmed by determining molecular mass by electrospray mass spectrometry.

5 *Immunization methods.*

Mice were immunized with 50 μ g of the indicated peptide in incomplete Freund's adjuvant (1SA51, Seppic Inc., Paris France) at weeks 0, 3, and 7 and bled at weeks 2, (bleed 1 after boost 1), week 5 (bleed 2 after 10 boost 2) and week 8 (bleed 3 after boost 3). Immune responses were seen after bleed 2 in most animals and data are reported from bleeds 2 and 3.

Guinea pigs were immunized intranasally with 200 μ g of C4-V3 peptide in saline with 1 μ g of cholera toxin as adjuvant as described. Guinea pigs were immunized on day 0, day 14 and day 21 and serum samples before 15 and 1 week following each immunization obtained by cardiac puncture.

6 *ELISA Assay.*

Anti-HIV env peptide ELISA assays were performed as previously described (Haynes et al, J. Immunol. 151:1646-1653 (1993), Haynes et al, AID Res. Human Retroviruses 11:211-221 (1995)).

20 *Splenocyte Proliferation Assay.*

Mouse splenocyte proliferation assay using 3 H-thymidine incorporation was performed as previously described (Haynes et al, AID Res. Human Retroviruses 11:211-221 (1995)).

7 *Neutralizing Antibody Assays.*

Assays for ability of anti-HIV antisera to neutralize HIV were 25 performed as described (Palker et al, J. Immunol. 142:3612-3619 (1989), Haynes et al, Trans. Am. Assoc. Physician 106:31-41 (1993), Haynes et al, J.

Immunol. 151:1646-1653 (1993), Haynes et al, AID Res. Human Retroviruses 11:211-221 (1995)).

NMR spectroscopy.

Peptides were dissolved to 4 mM in a solution of 90% $^1\text{H}_2\text{O}$, 10% $^2\text{H}_2\text{O}$,
5 20 mM NaCl, 5 mM KH₂PO₄, 1 mM sodium azide, 0.5 mM sodium 3-(trimethylsilyl) propionate, at a pH of 4.2. The methyl resonance of the latter component served as a chemical shift reference.

Spectra of samples prepared in this way were acquired with a Varian Unity 500 MHz spectrometer at a temperature of 278 K. The lock signal was
10 from deuterium in the sample. The following two-dimensional spectra were obtained: (a) double-quantum-filtered correlation spectroscopy (DQF-COSY) (Piantini et al, J. Am. Chem. Soc. 104:6800-6801 (1982), Rance et al, Biochem. Biophys. Res. Commun. 117:479-485 (1983)); (b) total correlation spectroscopy (TOCSY) (Bax et al, J. Magn. Reson. 65:355-360 (1985), Levitt et al, J. Magn. Reson. 47:328-330 (1982)) with a mixing time of 150 ins; and
15 (c) nuclear Overhauser exchange spectroscopy (NOESY) (Jeener et al, J. Phys. Chem. 71:4546-4553 (1979)), with a mixing time of 300 ins. Water resonance was suppressed by selective saturation during the relaxation delay, and, for NOESY, during the mixing period. The spectral width was 6700 Hz, with the
20 indirectly acquired dimension collected as 750 (COSY), 512 (TOCSY), or 350 (NOESY) complex increments; and the directly acquired dimension containing 1024 complex points. Data were processed with FELIX 2.3 software (Biosym, San Diego, CA). Directly acquired free-induction decays were corrected for base-line offset. Decays in both dimensions were multiplied
25 by a sinebell-squared function (phase shifted by 75°) and zero-filled to 2048 points before Fourier-transformation.

Peptide Membrane Binding Assay.

Peptides at 100ng/ml were incubated with 10⁶ peripheral blood mononuclear cells for 1 hour at 4°C, washed x3 with phosphate buffered saline

PHz 7.0, contained 0.1% sodium azide, then incubated guinea pig anti-HIV 89.6 V3 antisera (xlhr) (Liao et al, J. Virol. 74:254-263 (2000)), wash as above and then incubated with FITC-conjugated goat anti-guinea pig IgG. After a final wash as alone, the cells were analyzed for the relative amount of peptide bound to either PB lymphocytes or PB monocytes as reflected in the mean fluorescent channel (MFC) of reactivity of the anti-HIV 89.6 V3 antisera.

Results

Anti-gp120 V3 Antibody Responses Following Immunization of Mice With C4-V3RF, C4_{E9V}-V3RF(A), C4_{E9G}- V3RF(A) and C4_{K12E}- V3RF(A) Peptides.

First, the ability of C4-V3HIVRF variants to modulate the immunogenicity of the peptide with regard to antibodies to the V3 portion of the C4-V3 immunogen were assayed. The results (Figure 1, Table 2) show differences among the four peptides in their ability to induce anti-HIVRF V3 antibody responses. Sera from C4_{E9V}-V3RF(A)-immunized mice had a log 10 higher anti-V3 antibody titer than either mice immunized with the native C4-V3RF(A) peptide or the C4E9V-V3RF(A) peptide variant. After one immunization, no anti-V3RF antibody response was seen in mice immunized with either C4-V3RF(A), C4_{E9G}-V3RF(A), or C4_{K12E}-V3RF(A) peptides. However, after only one immunization with 50µg of the C4_{E9V}-V3 peptide, the geometric mean titer to V3RF(A) peptide was 1:5012 (n =3 mice), with titers of 1:3200, 1:3200 and 1:12,800 in each of the three mice tested, respectively. Thus, the E9V C4-V3RF(A) variant induced a higher titer and earlier anti-gp 120 V3 antibody responses than the other C4-V3RF(A) peptides tested. After 2 boosts, C4_{E9V}-V3RF(A)-immunized mice had 2 logs higher anti-V3 antibody responses than did C4-V3RF(A) immunized mice (Figure 1, Table 2).

TABLE 2
Comparison of the Ability of C4-V3 Peptides To Induce HIV gp120 Anti-C4 and Anti-V3 Antibodies in Balb/c Mice

Peptide Immunogen	Number of Animals		Peptide on Plate in ELISA For Anti-Peptide Antibody Assay			
	C4	V3RF(A)	C4-V3RF(A)	C4E9G-V3RF(A)	C4E9V-V3RF(A)	C412EV3RF(A)
Geometric Mean Titer						
C4-V3RF(A)	6	2	1,584	2,239	1,195	1,584
C4 _{E9G} -V3RF(A)	6	2	6,310	7,079	5,623	3,162
C4 _{E9V} -V3RF(A)	5	14	151,356	131,825	87,096	87,096
C4 _{K12E} -V3RF(A)	6	1	8	8	1	3

5.

Data represent the reciprocal of endpoint dilutions at which the E/C was 3.0 in anti-peptide ELISA after two immunizations.

The C_{4K12E}-V3RF(A) peptide variant induced anti-V3 antibody responses 3 logs lower than the C4-V3RF(A) peptide after 2 immunizations (Figure 1, Table 2). Thus, single amino-acid replacements in the C4 T helper region had extraordinary effects on immunogenicity of the HIVRF gp120 V3 domain.

Comparison of the Ability of C4-V3RF(A) Peptides to Induce Anti-HIVgp120 Peptide 3H-Thymidine Incorporation in Splenocytes From Naive and Peptide-Immunized Mice.

Next, C4-V3 peptides were tested for their ability to stimulate proliferation of splenocytes from peptide-immunized mice. Balb/c mice were sacrificed after the third peptide immunization and their splenocytes assayed for the ability to proliferate to PHA and to each peptide type (Table 3). It was found that C4-V3RF(A), C_{4E9V}-V3RF(A), and C_{4K12E}-V3RF(A) peptides all induced *in vitro* proliferative responses to the immunizing peptides, whereas the C_{4E9G}-V3RF(A) variant peptide did not induce proliferative responses in E9G-primed mice significantly over responses of naive mice (Table 3). Regarding the ability of the E9V peptide variant to induce earlier and greater anti-V3 antibody responses compared to the other peptides tested, the C_{4E9V}-V3RF(A) peptide-primed splenocytes for proliferation to the immunizing peptide only minimally better than did each of the other three peptides (Table 3). Thus, altered induction of T helper cell proliferative responses did not explain the differences in peptide immunogenicity.

TABLE 3

Comparison of the Ability of C4-V3 Peptides To Induce Anti-HIV gp120 Peptide ^3H -Thymidine Incorporation in Splenocytes from Naïve and Immunized Mice

Peptide Immunogen	N	Peptide Used As Stimulator in ^3H -Thymidine Incorporation Assay			C4 _{E9V} -V3RF(A)	C4 _{K12E} -V3RF(A)
		C4	V3RF(A)	C4-V3RF(A)		
	Mean \pm SEM	CPM per 10^6 Splenocytes in Culture				
None (Naïve)	6	613 \pm 322	408 \pm 140	149 \pm 84	114 \pm 85	74 \pm 47
Balb/c						
C4-V3RF(A)	6	2,289 \pm	955 \pm 353	8,390 \pm 1,424	8,067 \pm 1,728	6,242 \pm 1,787
		1,332	a			
C4 _{E9G} -V3RF(A)	6	408 \pm 95	708 \pm 325	2,103 \pm 1,170	3,559 \pm 2,310 b	988 \pm 340
C4 _{E9V} -V3RF(A)	5	84 \pm 52	1,463 \pm 473	933 \pm 4,528	11,743 \pm 3,830	24,824 \pm 5,581 c
C4 _{K12E} -V3RF(A)	6	3,430 \pm	4,417 \pm 2,217	8,670 \pm 3,865	13,237 \pm 8,563	7,513 \pm 2,951
		2,796				12,644 \pm 4,138 d

Data represent peak ^3H -thymidine responses at 7 days.

CPM = CPM experimental – experimental – experimental control.

^ap < .001 vs naïve mice; p = NS vs C4-V3RF(A) or C4K12E-V3RF(A) stimulated C4K12E-V3RF(A) immunized splenocytes.

^bp = NS vs naïve mice.

^cp < .001 vs naïve mice.

^dp < .02 vs naïve mice.

The lower antibody titer induced by the C_{4K12E}-V3 peptide against V3RF(A) was not an artifact attributable to lack of ability of the V3 peptide not binding to the ELISA plate, as sera from C_{4E9V}-V3RF(A)-induced antisera had high reactivity to the V3RF(A) peptide on the ELISA plate. Similarly, the C_{4K12E}-V3RF(A) peptide could bind anti-V3RF antibody, as multiple antisera raised against C4-V3 peptides bound the C_{4K12E}-V3 variant (Table 2).

Antibody levels to the C4 region were also tested. The C4 region induced only a minimal antibody response compared to the V3 region, with all the C4-V3 peptides tested (Table 2).

Anti-gp 120 V3 Antibody Responses Following Immunization of Guinea Pigs.

Next, 2 guinea pigs were immunized each with 200 μ g of C4-V3RF(A), C_{4E9G}-V3 RF(A), C_{4E9V}-V3 RF(A) or C_{4K12E}-V3 RF(A) peptide intranasally with 1 μ g cholera toxin adjuvant in saline. Intranasal immunization of peptides with cholera toxin has been previously shown to result in CTL and titers of anti-peptide antibody similar in levels to titers induced by initial antigens administered subcutaneously or intramuscularly in oil in water adjuvants such as complete and incomplete Freund's adjuvant. In addition, it was desirable to determine the ability of C4-V3 peptides in an aqueous solution (such as in saline for intranasal immunization) to induce anti-HIV antibody responses in order to correlate reactivity of antibodies generated against peptide in an aqueous adjuvant with peptide conformers solved in an aqueous solution. Finally, there was interest in determining if the amino acid substitutions in the C4 region conferred on the C4-V3 peptides the same pattern of immunogenicity as seen in oil in water adjuvant in mice.

It was found that after 2 immunizations the C4-V3 RF(A) peptide induced a mean anti-HIV peptide antibody titer of 3981, peptide induced titers of 1 log (GMT = 31,623) higher. As in mice, substituting the Glu (E) for Lys (K) at position 12 in the C4 peptide abrogated peptide immunogenicity in guinea pigs (GMT = 16) (Table 4).

TABLE 4

Titers of C4-V3 HIV Envelope Antibodies Induced by
C4-V3RF(A) Peptides in Guinea Pigs

<u>Immunizing Peptide</u>	<u>Titer Against Immunizing Peptide*</u>
C4-V3RF(A)	3,981
C4-E9G-V3RF(A)	2,818
C4-E9V-V3RF(A)	31,623
C4-K12E-V3RF(A)	16

*Data represent the mean titers from 2 animals after 2-3 immunizations intranasally with 400ug of the indicated peptide formulated in saline with cholera toxin as an adjuvant.

Ability of Antibodies Against C4- V3 Peptides to Induce Neutralizing Antibodies.

In order to induce high levels of neutralizing antibodies with C4-V3 peptides, usually 5 immunizations are given (Palker et al, J. Immunol. 142:3612-3619 (1989), Haynes et al, J. Immunol. 151:1646-1653 (1993), Palker et al, Proc. Natl. Acad. Sci. USA 85:1932-1936 (1988), Liao et al, J. Virol. 74:254-263 (2000)). The guinea pig sera from the experiment presented in Table 4 were tested for ability to neutralize HIVRF. It was found that one sera from the C4-V3RF(A)-immunized animals (after 3 injections) had a neutralizing antibody titer of 1:40 against HIVRF, while one animal of the C4_{E9V}-V3RF(A)-injected animals had a neutralizing titer of 1:340 after only 2 injections. Thus, antibodies induced by the C4_{E9V}-V3RF(A) peptide can bind to native gp120 and neutralize HIVRF.

Inability of the C4-E9V-RF(A) Sera to Bind to gp120 from HIV_{III}B.

The V3 loop sequence of HIV_{III}B is different from that of HIVRF, and thus HIVRF anti-V3 neutralizing antibodies do not neutralize HIV_{III}B. To determine if any antibodies were generated by any of the C4-V3RF(A) variant peptides, all the mouse sera in Table 2 were tested, as were the guinea pig sera in Table 4, for the ability to bind to native recombinant HIV_{III}B gp120 in ELISA. Since anti-HIVRF V3 antibodies do not bind to the HIV_{III}B V3 loop, any binding activity of these anti-C4-V3 sera would be to the C4 region of HIV_{III}B, which is conserved between HIV_{III}B and HIVRF. No binding of any mouse or guinea pig anti-C4-V3 sera to HIV_{III}B gp120 was seen, indicating the inability of these peptides to induce antibodies against the native gp120 C4 region.

Conformational Propensities of C4- V3 RF Sequence Variants in Aqueous Solution.

Next, the peptides were examined by NMR to determine whether conformational changes had been induced by amino-acid sequence alteration. It was hypothesized that specific amino-acid substitutions in the C4 segment would lead to a decrease in the tendency of this region to adopt transient helical conformations. To test this hypothesis, each of the four peptides, C4-V3RF and variants E9G, E9V and K12E, was subjected to ¹H NMR spectroscopy to assign resonances and to analyze nuclear Overhauser effects between hydrogen nuclei on separate residues.

Resonance assignments for nearly all ¹H were determined from TOCSY, DQF-COSY, and NOESY spectra by standard methods (Wuthrich, NMR of Proteins and Nucleic Acids, John Wiley and Sons, New York (1986)), and are shown in Figure 2. The value of the chemical shift for a main-chain ¹H, for example, the a carbon C^aH, is correlated with secondary structure in the case of proteins or well structured peptides (Wishart et al, J. Mol. Biol. 222:311-333 (1991)). Hence, strong tendencies among C4-V3RF

peptides to adopt secondary structure in solution may be manifested in chemical shift values. This was examined by calculating for each peptide the difference in chemical shift between the C-H of each residue and a shift value representing the average for all secondary structures in proteins (Wishart et al, J. Mol. Biol. 222:311-333 (1991)). In no peptide were there stretches of sequence with high or low values of the chemical shift difference that would be evidence of stable secondary structure, for example helix or β strand.

NMR parameters such as chemical shift and coupling constants are often insensitive indicators of weak preferences for particular conformations since their values are the average of the entire population, thus obscuring the contribution of a slight bias for populating certain conformations. The nuclear Overhauser effect (NOE) is often more sensitive at revealing conformational propensities because it may give rise to a unique signal, although weak, on a background consisting only of random noise. Hence, NOESY spectra of C4-V3RF and its variants were characterized to identify each signal and evaluate its relative intensity. Sequential and medium range NOEs involving main-chain NH or CaH are listed in Figure 2. These NOEs and the possible conformational propensities they represent are discussed as follows for C4_{E9G}-V3RF(A) and C4_{E9V}-V3RF(A). Variant C4_{K12E}-V3RF(A)K12E is discussed separately below because it was studied under different conditions.

In terms of overall conformation, all four peptides showed NOE patterns suggesting no tendency to adopt stable structure. For example, sequential d_aN(*i*, *i*+1) and dNN(*i*, *i*+1) NOEs were usually both present for each sequential pair of residues, with the former typically more intense, indicating that *f* and *j* main-chain dihedral bond angles varied and maintained on average an extended conformation (Dyson et al, Ann. Rev. Biophys. Chem. 20:519-538 (1991)). Also the absence of long range NOEs [(*i*, *i*+5) or greater] and the few and generally weak medium-range NOEs suggested no significant population of higher order structure.

However, the fact that some medium range NOEs were detected is evidence of propensity to adopt non-random conformations in certain regions (Dyson et al, Ann. Rev. Biophys. Chem. 20:519-538 (1991)). Although only one mixing time was used for NOESY spectra (300 ins), previous studies of a related C4-V3 RF peptide (de Lorimier et al, Biochemistry 33:2055-2062 (1994)) showed that medium range NOEs were still observable at shorter (75 and 150 ins) mixing times. Hence, the NOEs indicating medium range interactions are not likely due to spin-diffusion.

Within the C4 segment C4-V3RF and C4_{E9V}-V3RF(A) showed numerous medium range NOEs which are consistent with a tendency of this region to populate nascent helical conformations. The presence of contiguous or overlapping *daN*(*i,i+2*) NOEs from Trp⁷to Tyr¹⁵ (C4-V3RF) and from Ile⁴ to Lys¹² (E9V) indicates a propensity for nascent helical turns in these regions (Dyson et al, Ann. Rev. Biophys. Chem. 20:519-538 (1991), Dyson et al, J. Mol. Biol. 201:201-217 (1988)). A *dNN*(*i,i+2*) NOE in this region in C4-V3 RF (between Lys¹² and Met¹⁴) is also consistent with main-chain f and j dihedral angles representative of helical turns (Dyson et al, Ann. Rev. Biophys. Chem. 20:519-538 (1991)). C4-V3 RF shows three consecutive *daN*(*i,i+3*) NOEs from residues Val¹⁰ to Tyr¹⁵, which is highly indicative of full helical turns. The presence of equivalent NOEs in E9V could not be ascertained due to overlap with other NOEs. However both C4-V3RF and E9V show two *dab*(*i,i+3*) NOEs, between Val¹⁰ and Ala¹³ and between Ala¹³ and Met¹⁴. This type of NOE is also highly suggestive of full helical turns in these regions of C4.

Variant C4_{E9G}-V3RF(A)on the other hand showed no evidence, in terms of medium range NOEs, for preferential population of certain conformations in C4. This absence of medium range NOEs was not due merely to ambiguities caused by signal overlap, because there were at least five positions where an NOE was unambiguously absent in C4_{E9G}-V3RF(A),

but present in the parent peptide C4-V3 RF. Thus, the E to G substitution in the C4 peptide appeared to prevent helical conformer formation in the peptide.

In the V3 segment of the three peptides, C4-V3 RF, C4_{E9G}-V3RF(A) and C4_{E9V}-V3RF(A), were medium range NOEs suggesting preferred solution conformations in certain RE regions. All three peptides showed evidence of a reverse turn in the sequence Arg¹⁸-Pro¹⁹-Asn²⁰-Asn²¹, where these residues comprised positions 1 to 4, respectively, of the turn. The NOE pattern consistent with a reverse turn included a weak *dNd(i,i+1)* between Arg¹⁸ and Pro¹⁹, undetectable *ddN(i,i+1)* between Pro¹⁹ and Asn²¹, weak *dad(i,i+1)* between Arg¹⁸ and Pro¹⁹, strong *daN(i,I+1)* between Pro¹⁹ and Asn²⁰, and detectable *daN(i,i+2)* between Pro¹⁹ and Asn²¹ (Dyson et al, J. Mol. Biol. 201:161-200 (1988)). The detection of the weak *dNd(i,i+1)* NOE (Arg¹⁸ to Pro¹⁹) suggested that a Type I turn may be the preferred conformation (Dyson et al, J. Mol. Biol. 201:161-200 (1988)).

All three peptides also showed evidence of preferred conformers at the sequence Ser²⁶-Ile²⁷-Thr²⁸-Lys²⁹. There were two consecutive *daN(i,i+2)* NOEs, between Ser²⁶ and Thr²⁸ and between Ile²⁷ and Lys²⁹, as well as medium range NOEs not shown in Figure 2. The latter included a *dbN(i,i+2)* NOE between Ser²⁶ and Thr²⁸, and a *dba(i,i+2)* NOE between these same residues. The conformational preferences giving rise to these NOEs did not fit a typical secondary structure, and suggested an unusual turn that placed the side-chain of Ser²⁶ in close proximity to the main-chain groups of Thr²⁸. This type of conformation has been described as a kink in the context of a helical region (Osterhout et al, Biochemistry 28:7059-7064 (1989)).

A third conformational feature in the V3 segments of C4-V3RF, C4_{E9V}-V3RF(A) and C4_{E9G}-V3RF(A) occurred in the sequence Gly³⁰-Pro³¹-Gly³²-Arg³³. In E9G the NOEs between these residues resembled the pattern described above that was consistent with a reverse turn (Dyson et al, J. Mol. Biol. 201:161-200 (1988)). This included a weak *dNd(i,i+1)* NOE between Gly³⁰ and Pro³¹, a weak *ddN(i,i-I-i)* NOE between Pro³¹ and Gly³², a weak *dad(i,i+1)* NOE between Gly³⁰

and Pro³¹, a strong *daN(i,i+1)* NOE between Pro³¹ and Gly³², and a detectable *daN(i,i+2)* NOE between Pro³¹ and Arg³³. In the C4-V3RF peptide, the pattern of *(i,i+1)* NOE intensities was the same but no *daN(i,i+2)* NOE was detected between Pro³¹ and Arg³³. Instead a *daN(i,i+2)* NOE was detected between Gly³⁰ and Gly³². And in C4-E9V V3RF, both *daN(i,i+2)* NOEs, Gly³⁰ to Gly³² and Pro³¹ to Arg³³, were detected. These data raised the possibility that two independent turn-like conformational preferences occurred in this region of V3. The fact that a Pro³¹-Arg³³ *daN(i,i+2)* NOE was unambiguously absent in C4-V3RF, and that a *daN(i,I+2)* NOE between Gly³⁰ and Gly³² was also unambiguously absent in C4_{E9G}-V3RF(A), in spite of sequence identity in all three peptides, may be related to the weak intensity of these NOEs. Being close to the level of noise intensity, there is a possibility that one or both NOE signals on either side of the spectrum will not be detected, thus disallowing the given NOE to be scored as such.

Another region in V3 where conformational preferences could be inferred from NOEs occurs in residues Val³⁴-Ile³⁵-Tyr³⁶. In all three peptides NOEs were observed between the upfield methyl resonance (~0.67 ppm) of Val³⁴ and the ring hydrogens, both dH and eH, of Tyr³⁶. Weaker NOEs are also seen between the downfield methyl resonance (~0.89 ppm) of Val³⁴ and the ring hydrogens of Tyr³⁶. Further evidence of close proximity between the side-chains of Val³⁴ and Tyr³⁶ was the fact that the two methyl resonances of the former had disparate chemical shifts, compared to Val¹⁰, consistent with a ring-current shift induced by the aromatic side-chain of Tyr. One peptide, C4-V3RF(A) had another NOE in this region, *daN(i,i+2)* between Ile³⁵ and Ala³⁷, that was unambiguously absent in the C4_{E9G}-V3RF(A) and C4_{E9V}-V3RF(A) peptides. This observation likely represented a poorly populated conformation, perhaps related to that which gives rise to the Val³⁴-Tyr³⁶ side-chain interaction, or from an independent conformational propensity.

Substitution of Lys¹² with Glu yielded a poorly immunogenic peptide (C4_{K12E}-V3RF(A)) that, interestingly had solution properties different from the

other three peptides studied. Under the conditions used for NMR studies of other C4-V3 peptides, the solution of the C4_{K12E}-V3RF(A) peptides was highly viscous, and viscosity increased with pH in the vicinity of pH 4, implicating ionization of the Glu¹² side-chain in this phenomenon. NMR spectra of K12E at 278 K in aqueous buffer showed a much lower signal-to-noise ratio than the other three peptides. Increasing the temperature to 318 K or decreasing the pH to 3.5 yielded improved but still inadequate signal. Suitably high signal for resonance assignment and NOE analysis was obtained at 318 K, pH 3.5, 20% v/v trifluoroethanol (*d*₃). Even under this condition the NOEs for the C4_{K12E}-V3RF(A) were less intense than for other peptides.

NOE connectivities in the C4 segment of C4_{K12E}-V3RF(A) (Fig. 2) show evidence of nascent helical turns in the region between Ile³ and Gly¹¹ as inferred from *dNN*(*i, i+2*) and *daN*(*i, i+2*) NOEs. The stretch from Val¹⁰ to Thr¹⁷ has two *daN*(*i, i+3*) and two *dab*(*i, i+3*) NOEs suggesting the presence of a significant population with full helical turns. Within the V3 segment only two medium range NOEs are observed, both *daN*(*i, i+2*). Neither corresponds to NOEs observed in the other three peptides, but both NOEs involve residues of the Ser²⁶-Ile²⁷-Thr²⁸ sequence, for which there is evidence of conformational preferences in the other three peptides. A *dbN*(*i, i+2*) NOE between Ser²⁶ and Thr²⁸, observed in C4_{E9V}-V3RF(A) and C4_{E9G}-V3RF(A), is also observed in the K12E peptide. Also observed are NOEs between the side-chains of Val³⁴ and Tyr³⁶. Hence the conformations giving rise to these two features are at least partially preserved under the solution conditions employed for K12E. Differences in the V3 segment between K12E and all of the other three peptides include the absence of detectable *daN*(*i, i+2*) NOE between Pro¹⁹ and Asn²¹ and between Ser²⁶ and Thr²⁸. The failure to detect these NOEs may be due to the overall weaker signals of this sample, or to depopulation of the relevant conformations by the solution conditions.

EXAMPLE 2

The peptides in Table 7 have been studied in groups of 5 peptides as indicated in Table 9, and each group of 5 peptides has been injected into each of three guinea pigs in Freund's complete then incomplete adjuvant. After 4 immunizations, the animals were bled, and heat inactivated serum was pooled from each animal or tested separately as indicated in Table 8, for the ability to neutralize HIV. Single numbers per group indicate that the results are those of pooled sera from the group. Individual results per animal indicate that each serum was tested individually. Table 8 shows that all the sera neutralized to varying degrees the T cell line adapted HIV isolate MN and poorly neutralized the TCLA HIV isolate IIIB. Regarding the rest of the isolates in Table 8, all of which are HIV primary isolates (89.6, BAL ADA, SF162, 5768, QH0515, PVO, JRFL, BX08, 6101, SS1196), Group C sera from C4-V3 subtype B peptides neutralized 4/11 (36%) and Group F sera from subtype B peptides neutralized 5/11 primary isolates (45%). Figure 4 shows that for the HIV CCR5 utilizing primary isolate, BAL, that the individual peptides in the 5-valent mixture absorbed out the neutralizing activity against HIV BAL to varying degrees, whereas the mixture of all the peptides completely absorbed out the neutralizing activity.

Neutralization Of HIV-1 Isolates By Sera From Guinea Pigs Immunized With C4-V3 Clade B Peptides

Animal	ImmunoGen	HIV-1B	HIV-1B	STLV99/6#	STLV99/6#	IVIIV1* ADA*	SF162*	5168*	2110515*	PWp* MN1*	MN8*	6101*	551196*
477	A	2,238	0	96	0	0	90	0	0	0	0	0	0
478	A	1,357	0	NA	35	0	0	0	0	0	0	0	0
479	A	4,612	68	NA	0	0	0	0	0	0	0	0	0
480	B	1,158	0	NA	0	0	0	0	0	0	0	0	0
481	B	7,714	0	NA	77	84	0	96	0	0	0	0	0
482	B	4,241	0	62	0	0	0	0	0	0	0	0	0
483	C	969	0	112	0	95	0	0	0	0	0	0	0
484	C	806	0	20	97	89	0	99	0	0	0	0	0
485	C	512	0	226	0	80	0	0	0	0	0	0	0
486	D	1,488	0	NA	0	0	0	0	0	0	0	0	0
487	D	2,184	0	NA	98	80	0	98	0	0	0	0	0
488	D	575	0	NA	0	0	0	0	0	0	0	0	0
489	E	3,721	0	NA	0	88	0	0	0	0	0	0	0
490	E	NA	0	NA	255	0	0	92	0	0	0	0	0
491	E	519	0	NA	0	81	0	0	0	0	0	0	0
492	F	NA	0	NA	0	NA	0	0	0	0	0	0	0
493	F	910	0	NA	0	91	0	84	0	0	0	0	0
494	F	1,159	35	NA	0	NA	0	0	0	0	0	0	0

#Assay titers are reciprocal serum dilutions at which 50% of MT-2 cells were protected from virus-induced killing as measured by neutralized uptake. % reduction in p24 synthesis relative to the amount of p24 induced in the presence of corresponding peptide. Values >90% are positive. NA = Not available.

6. Pig Immunization Protocol Part 2

Peptide Number	Peptide Sequence	Code: GP#
C4 V1 091	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTK	A
C4 V1 1185	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTG	A
C4 V1 1429	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTPG	A
C4 V1 1781	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTG	A
C4 V1 1921	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTG	A
C4 V1 3171	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTG	B
C4 V1 3629	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTG	0
C4 V1 5170	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTG	B
C4 V1 5329	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTG	B
C4 V1 4676	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTG	B
C4 V1 6916	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTG	C
C4 V1 7218	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYATG	C
C4 V1 1018	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTG	C
C4 V1 6219	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTH	C
C4 V1 7417	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTG	C
C4 V1 8715	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTG	D
C4 V1 1011	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTG	D
C4 V1 8614	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYAT	D
C4 V1 8515	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTG	D
C4 V1 1229	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTG	D
C4 V1 1706	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTG	E
C4 V1 1468	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTK	E
C4 V1 1637	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTG	E
C4 V1 1759	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTTG	F
C4 V1 1627	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTG	F
C4 V1 1962	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYAT	F
C4 V1 1448	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTT	F
C4 V1 1682	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYATE	F
C4 V1 5132	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTG	F
C4 V1 1481	KQI IIAWQVVKHAYA- RPHNITRKS IIGPGCHAFYTG	F

It is important to be able to use T helper determinants with the V3 portion of the peptides shown in Table 7, both to expand the T helper activity in the immunogen, and in case any of the T helper peptides should be found to have any deleterious effects in the course of human trials. For example, it has recently been found *in vitro* that in culture of HIV and T cells, that the C4 portion of the C4-V3 peptide can augment HIV induced syncytium formation. However, peptides of this general design have been studied *in vitro* in HIV-infected humans (AIDS 12: 1291-1300, 1998) and no subjects developed a \geq 10 fold change in plasma HIV RNA levels from baseline. Moreover, the primary use of these peptides is as an immunogen in HIV- subjects as a preventive vaccine, and not in doses that one would consider for therapy, which would be in milligram amounts daily. A T helper determinant from HIV gag, termed GTH1 with the sequence of Y K R W I I L G L N K I V R M Y S has been conjugated to the V3 of HIV MN and found to induce anti-HIV MN titers of 1:3200. Similarly, GTH1 conjugated to a V3 sequence of a HIV primary isolate DU179 induced antibodies that neutralized HIV MN (1:192) and neutralized the HIV primary isolate JR-FL (90% p24 reduction in PBMC cultures). Thus, the GTH1 T helper sequence can substitute for the C4 sequence in the peptides in Table 7.

Finally, a panel of monovalent serum from individual guinea pigs immunized with each of the peptides in Table 7 has been screened. Whereas most of the peptides in the list only induced neutralizing antibodies that neutralized 0 to 6 out of 19 primary isolates, 5 peptides were found that neutralized from 14 to 19 out of 19 primary isolates tested. These peptides were C4-V3 36.29, C4-V3 34.29, C4-V3 62.19, C4-V3 74.17, and C4-V3 162.7. The sequences of these peptides are all listed in Table 7.

Thus, sufficient breadth has been observed both in mixtures of C4-V3 peptides and in select individual peptides for the immunogen to be practical with regard to induction of neutralizing antibodies against HIV primary isolates. By performing the same immunization studies with the similarly

designed HIV subtype (clade) C peptides in Table 6, that a similar immunogen(s) can be developed for HIV subtype C viruses.

While individual peptides can be used to achieve the breadth of neutralizing activity needed to protect against HIV primary isolates, advantageously, mixtures of multiple peptides are used, such as the combination of group C, or group F or the combination of C4-V3 36.29, C4-V3 34.29, C4-V3 62.19, C4-V3 74.17, and C4-V3 162.7 peptides described above.

EXAMPLE 3

HIV-1 Clade B V3-Based Polyvalent Immunogen

Anti-HIV gp120 V3 antibodies can neutralize some HIV primary isolates ((Hioe et al, Internat. Immunology 9:1281 (1997), Liao et al, J. Virol. 74:254 (2000), Karachmarov et al, AIDS Res. Human Retrovirol. 17:1737 (2001), Letvin et al, J. Virol. 75:4165 (2001)). The hypothesis for these studies was that sequence variation found among HIV primary isolates need not reflect the diversity of HIV serotypes, and antibodies can cross-react with groups of similar viruses. Data from comparison of NMR structures of several V3 loops and their immunogenicity patterns indicate that there are conserved higher order structures of the V3 that are similar in antigenicity regardless of primary amino acid heterogeneity (Vu et al, J. Virol. 73:746 (1999)).

1514 unique clade B V3 sequences in the Los Alamos National Laboratory HIV Database were analyzed by the following methods. Short antigenic domains were organized by protein similarity scores using maximum-linkage clustering (Korber et al, J. Virol. 68:6730 (1994)). This enabled visualization of clustering patterns as a dendritogram, and the splitting pattern in the dendritogram could be used to define clusters of related sequences. This method allows the use of several different amino acid scoring

schemes. The amino acid substitution matrix of Henikoff and Henikoff was used which was designed to give amino acid substitutions well tolerated in conserved protein structural elements a high score, and those that were not, a low score (Henikoff and Henikoff, Protein Structure Function and Genetics 17:49 (1993)). Based on these clustering patterns, a variant was selected that was most representative of each group. Excluded were very rare, highly divergent sequences, and favored were sequences found in many different individuals. This method allowed for most of the unique V3 sequences to be within one or two amino acids from at least one of the peptides in the cocktail. Thus, 1514 clade B V3 sequences were clustered into 30 groups. The consensus peptide of each group was synthesized, purified to homogeneity by HPLC and confirmed to be correct by mass spectrometry. Each peptide was immunized into a guinea pig (GP) in Incomplete Freunds Adjuvant (IFA), and each sera was tested after the fifth immunization by a single infection cycle neutralization assay preformed by ViroLogics, South San Francisco, CA, or by a fusion from without HIV fusion inhibition assay using aldrithiol-2 inactivated HIV_{ADA}, HIV_{MN} and HIV_{AD8} viroids (Rosio et al, J. Virol. 72:7992 (1998)).

The criteria established for acceptable neutralization of primary isolates was the ability of a serum to neutralize at least 25% of the HIV primary isolates tested. Using these criteria, 7 peptides were found that induced neutralizing antibodies against >25% of isolates tested. One of these peptides, peptide 62.19, neutralized 19/19 HIV primary isolates tested, even when the criteria were increased to greater than 80% neutralization vs. 50% neutralization (see Figure 5 and Table 11).

When the sequences of 6 peptides that induced no (0/19) neutralization of the 19 primary HIV isolates were evaluated, it was found that they were all unusual sequences at the tip of the V3 loop, with sequences such as GLGR, GPGG, GLGK, GLGL, and GLGR present (see Table 10). Only 1 of the 19 isolates tested had one of the these V3 sequences, a GPGG sequence, that was

not neutralized by the serum from the GPGG-immunized guinea pig. Therefore, one serologic defined group of Clade B HIV isolates may be defined by the primary amino acid sequences at the tip of the loop of GLGR, GPGG, GLGK, GLGL.

5

Table 10

**Sequences of Peptides That Induced No Neutralization
at 50% Inhibition (All Dilutions) Criteria**

GP No.	Peptide No.	V3 Sequence(s)
447	C4-V3 396.2	RPNNNTRRN <i>I</i> HIGL GRRFYAT
448	C4-V3 170.6	RPNNNTRRSVR <i>I</i> G PGGAMFRTG
451	C4-V3 23.38	RPIK I E KR IPL GLGKAFYTTK
458	C4-V3 51.23	RPSVNNTRRSIH MGLGRAFYTTG
404	C4-V3 57.20	RPNRHTGKSIRMG GLGLRAWHTTR
432	396.2/170.6	RRNIHIG LGR RF RRSVRI GPGGAM

10

15

20

41

Table 11

**Sequences of Peptides That Best Neutralized Clade B
Isolates at 50% Inhibition (All Dilutions) Criteria**

GP No.	Peptide No.	V3 Sequence(s)
436	69.18/146.8	RKSIR <u>I</u> GPGRAV RRRISIG <u>P</u> GRAF
442	1.481/85.15	RKSIH <u>I</u> GPGRAF RKSIH <u>A</u> PGRAF
460 (B)	C4-V3 36.29	RPNNNTRKG <u>I</u> HIGPGRTFFATG
465 (A)	C4-V3 11.85	RPNNNTRKSIN <u>I</u> GPGRAFYTTG
466 (A)	C4-V3 34.29	RPNNNTRKSI <u>Q</u> I <u>I</u> GPGRAFYTTG
467 (A)	C4-V3 1.481	RPNNNTRKSIH <u>I</u> GPGRAFYTTG
469 (C)	C4-V3 62.19	RPNNNTRKSIH <u>I</u> GPGRAFYATE
472 (C)	C4-V3 74.17	RPNNNTRKRMTL <u>G</u> PGKVFYTTG
475 (E)	C4-V3 162.7	RPGNNTRGSIHL <u>H</u> PGRKFYYSR

When the peptide sequences that induced neutralization of >25% of primary isolates were examined, it was found that the sequences were all similar and were all clustered around the Clade B V3 consensus sequence of IHIGPGRAFYTTG (see Table 11). However, not all peptides with this type of sequence induced good neutralizing antibodies—15 peptides had this type of sequence and did not induce good neutralizing antibodies. Thus, a “computer guided proteomic screen of the V3 loop” has been performed and V3 peptides have been identified that express higher order conformers that mirror the native functionally active motif of the V3 that is both available and capable of being bound by neutralizing antibodies. In particular, peptide 62.19 induced neutralizing antibodies against 19 of 19 HIV isolates.

Expression of the consensus B V3 sequences in Table 11, and expression of certain of the unusual V3 sequences in Table 10, can define a “bivalent” clade B immunogen for use world wide where those sequences are present in the resident HIV quasispecies, likewise, the sequences shown in Table 12. Table 12 shows full V3 consensus sequences for the V3 loops of the indicated

peptides. By placing these full length V3 loop sequences into a full length HIV envelope gp120 or gp160/gp140 molecule, the ability of these peptides to induce neutralizing activity is transferred to the HIV envelope containing these sequences. Thus, for example, for the artificially designed consensus of consensus HIV envelope with less divergence from other HIV isolates compared to native HIV envelopes (Gaschen et al, Science 296: 2354-2360 (2002)), inclusion of one of the V3 sequences in Table 12 that has been shown to induce neutralizing activity against HIV primary isolates would augment the ability of the consensus of consensus artificial envelope to induce neutralizing antibodies. Further, expressing the V3 sequences in Table 12 would augment their immunogenicity by combining the V3 with other neutralizing sites on an immunogen (the intact envelope monomer or trimer).

Immunization with a replicating vector, expressing partial or entire (C to C) segments of these V3 loops, can be used to induce long lasting immunity to HIV.

* * *

TABLE 12

Name of Peptide	Total Seq in Database	V3 Consensus Sequence
		Amino Acid Sequence
1.481	345	SVEINCTRPNNNTRKSIHIGPGRAYTTGEIIGDIRCAHCNISRA
62.18C	952	SVEINCTRPNNNTRKSIHIGPGRAYATERIIGDIRCAHCNISRT
62.19dT	-	SVEINCTRPNNNTRKSIHIGPGRAYATETTRIIGDIRCAHCNISRT
162.7	11	SVEINCTRPGNNTRGGSIHLHPGRKFYYSRGIIIGDIREAHCAINIP
170.5	7	SVEINCTRPNNNTRRSVRIGPGGAMFRTGDIIGDIRCAHCNLSRT
34.29	39	SIEINCTRPNNNTRKSIQIGPGRAYTTGEIIGDIRQAHCNLSRA
74.17	34	SVEINCTRPNNNTRKRMTLGPDKVFTTGIIIGDIRKAHCNISRA
396.2	2	SVAINCTRNNNTRRNHIIGLGRRFYATEIGOTKKACCNISRA
23.33	25	SVEINCTRPIKIERKRIPLGLGKAFYTTKQVGDIKCAHC
32.15	36	PVEINCTRPNNNTRRSIHIAPGRAYTTGQIIGDIRRAHCNISRT
57.2	21	TWVINCTRPNRHTGKSIRMGGLGRAWHTREIIGDIRKAYCTLNGT
36.29	46	SVNINCTRPNNNTRKGHIHIGPGRFFFATGDIIGDIRCAHCNLSRT CTRPNNNTRKSIHIGPGRAYTTGEIIGDIRCAHC
BAL V3		

All documents cited herein are incorporated in their entirety by reference.

WHAT IS CLAIMED IS:

1. A method of stabilizing a hairpin loop structure of a peptide that comprises residues of the V3 domain of gp120 and that includes a B cell neutralizing antibody epitope, said method comprising

adding or substituting on the C-terminal or N-terminal end of said hairpin loop one or more positively or negatively charged amino acids so that said stabilization is effected by hydrogen bonding between oppositely charged amino acids present at the C- and N-terminal ends of said hairpin loop.

2. The method according to claim 1 wherein at least one negatively charged amino acid is added on the C-terminal end of said loop.

3. The method according to claim 2 wherein from one to three negatively charged amino acids are added on the C-terminal end of said loop.

4. A peptide comprising a sequence selected from the group consisting of:

X¹-SIHIGPGRAFY-X²

X¹-SINIGPGRAFY-X²

X¹-GIHMGPGKAIY-X²

X¹-MTLGPVGKVFY-X²

X¹-GIHIGPGRTFF-X²

X¹-SIQIGPGRAFY-X².

wherein X¹ comprises at least two contiguous negatively or positively charged amino acids and X² comprises at least two contiguous amino acids bearing a charge opposite to that of the amino acids of X¹.

5. The peptide according to claim 4 wherein said peptide comprises an amino acid sequence set forth in Table 5.

6. A composition comprising a multiplicity of immunogenic polypeptides, each of said polypeptides comprising the peptide according to claim 4.

7. The composition according to claim 6 wherein said composition further comprises a carrier.

8. The composition according to claim 6 wherein said composition further comprises an adjuvant.

9. An isolated nucleic acid sequence encoding the peptide according to claim 4.

10. A vector comprising the nucleic acid sequence according to claim 9.

11. A composition comprising at least one nucleic acid sequence according to claim 9 and a carrier.

12. A method of inducing an immune response in a mammal comprising administering to said mammal an amount of at least one peptide according to claim 4 in an amount sufficient to effect said induction.

13. A method of inducing an immune response in a mammal comprising administering to said mammal an amount of at least one nucleic acid sequence according to claim 9 in an amount sufficient to effect said induction.

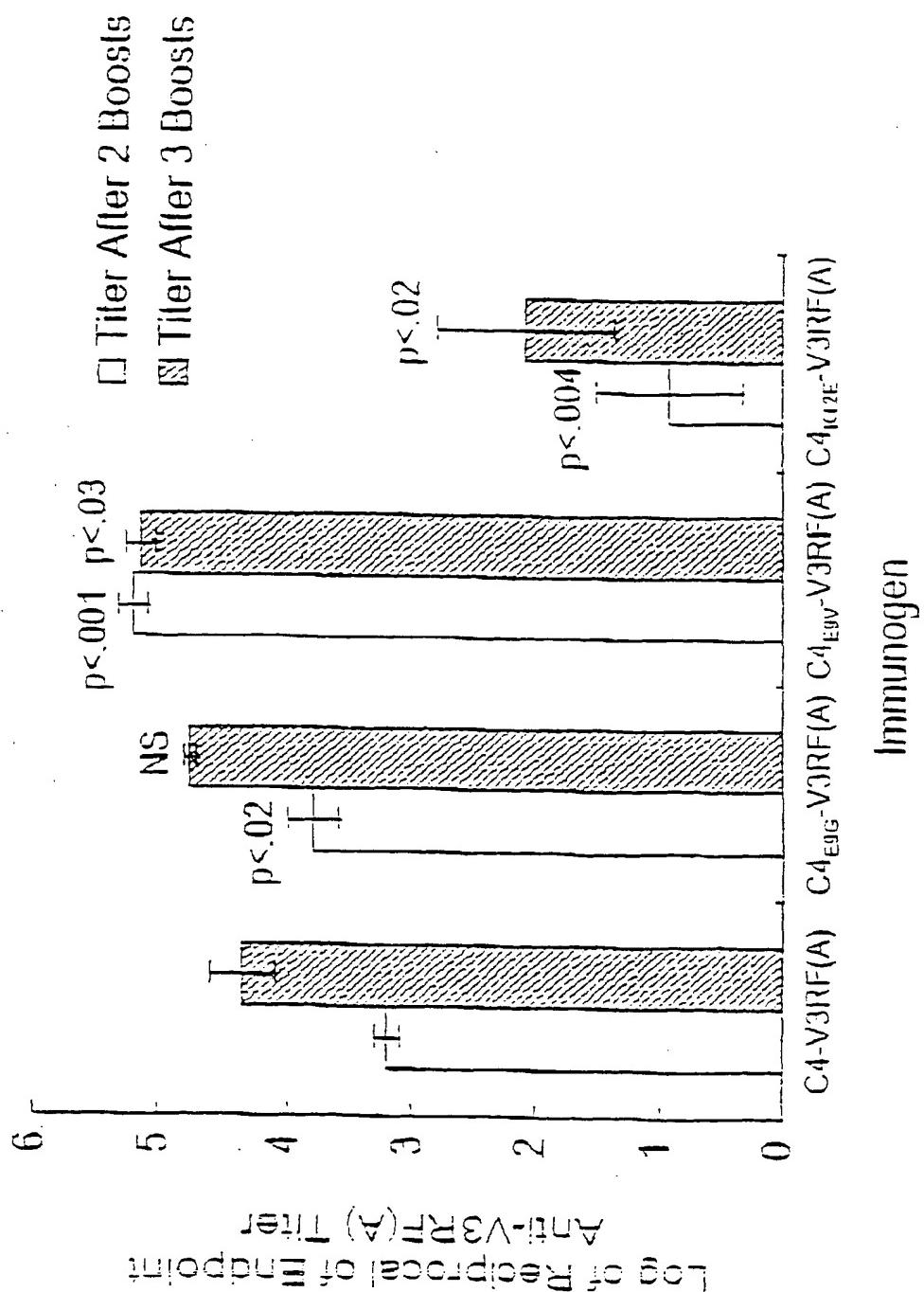
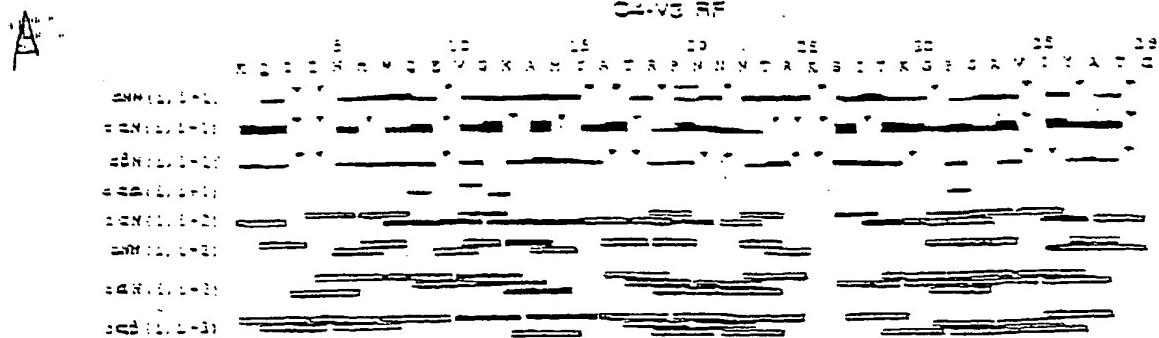


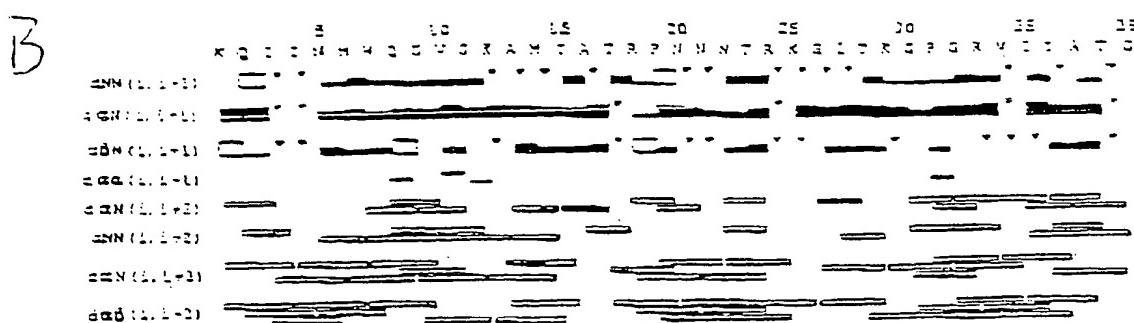
Figure 1

Figures -

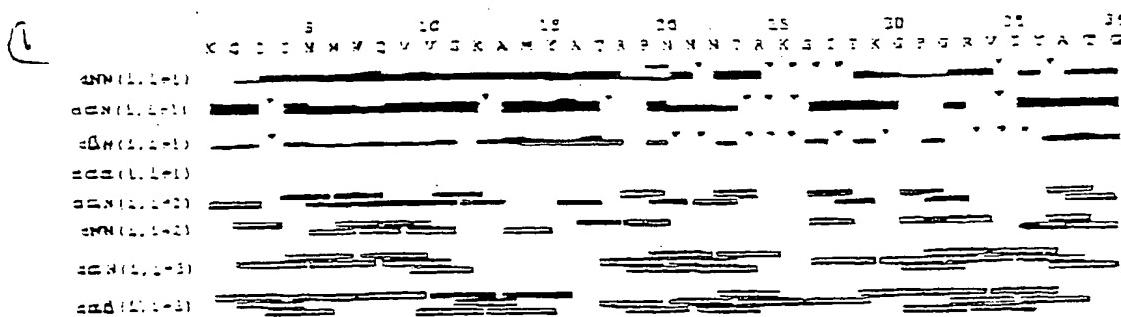
CA-VG RF



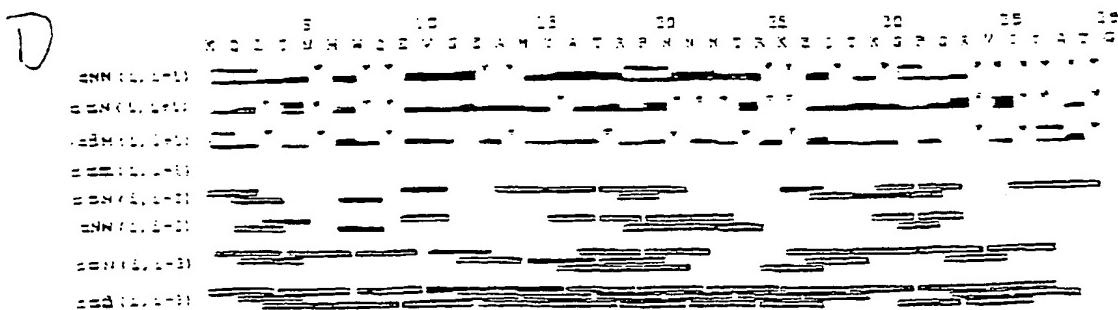
EG



ESV



K12E



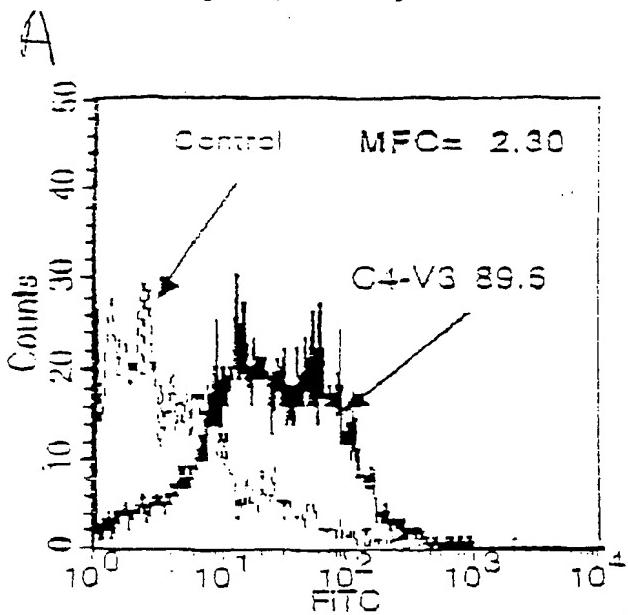
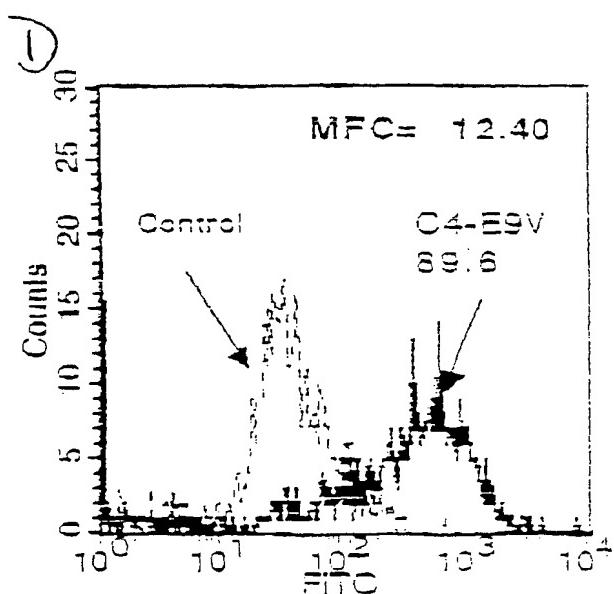
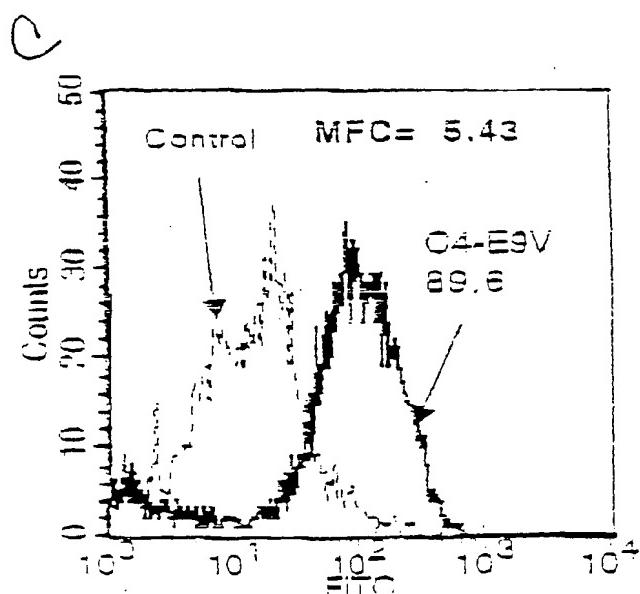
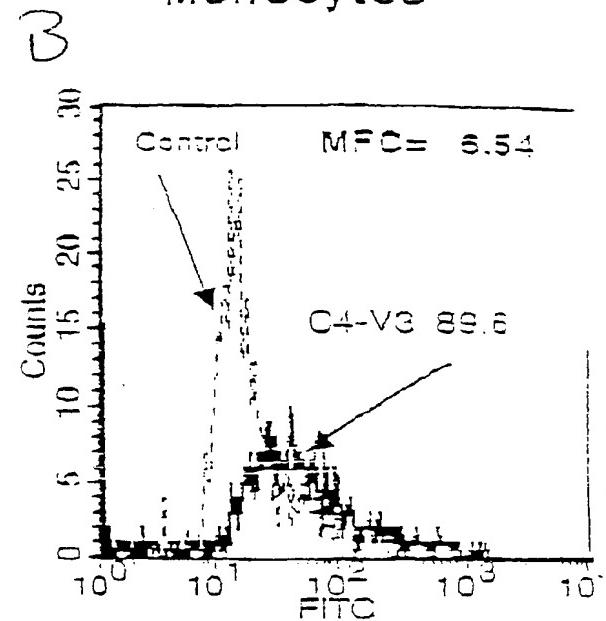
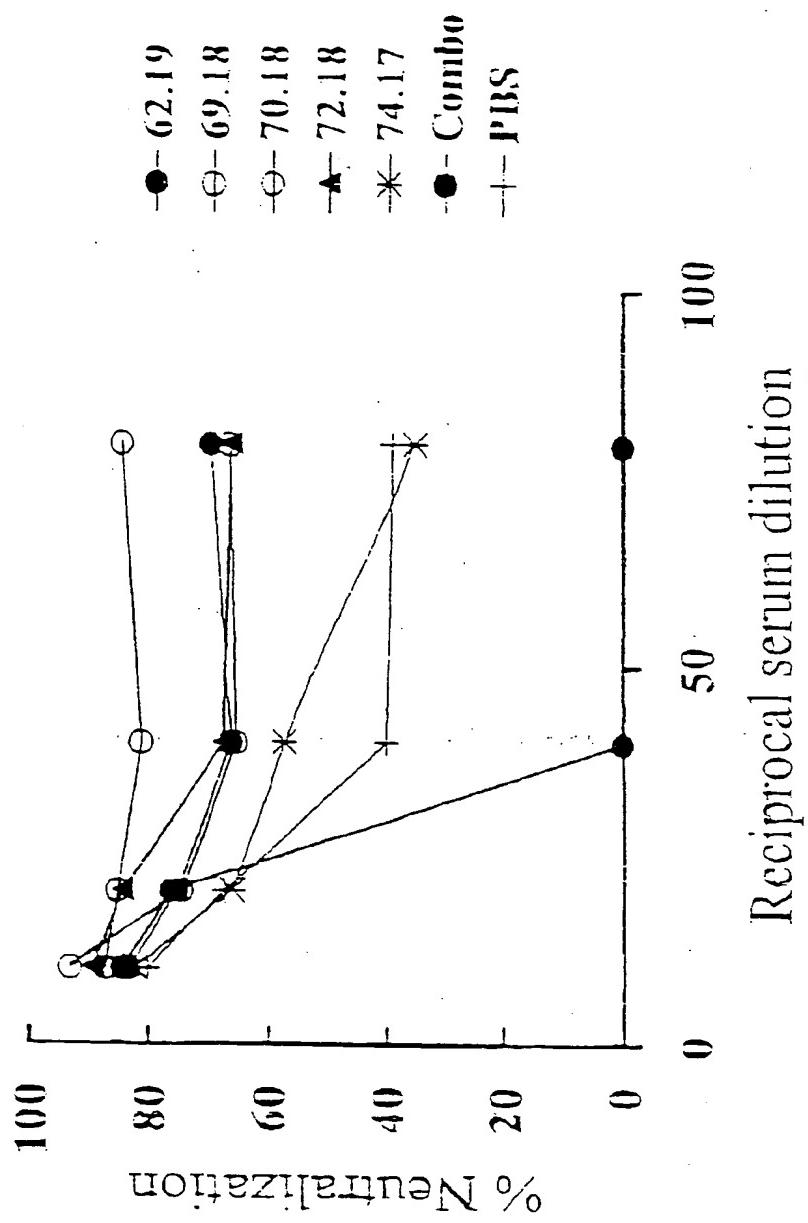
Lymphocytes**Monocytes**

Figure 61

Figure 4.

Neutralization of BAL in PBMC



C4-VB 62.19 (GP 469)

Sequences of 30 HIV Clade B C4-V3 Peptides

Figure 6

FIGURE 7

Strategy For Design of HIV gp120 V3 Immunogens With Higher Order Structures

FIGURE 7 (CONT'D) 1

Approaches to Develop Immunogens That Broadly Neutralize Primary Isolate HIV

- Variable Region Subunit Immunogens
- Constrained Envelope Immunogens
- Choice of “Best Env” Immunogen
 - Survey of a large panel of expressed Envs
 - Expression of consensus and ancestral “artificial” Envs

FIGURE 7 (CONT'D) 2

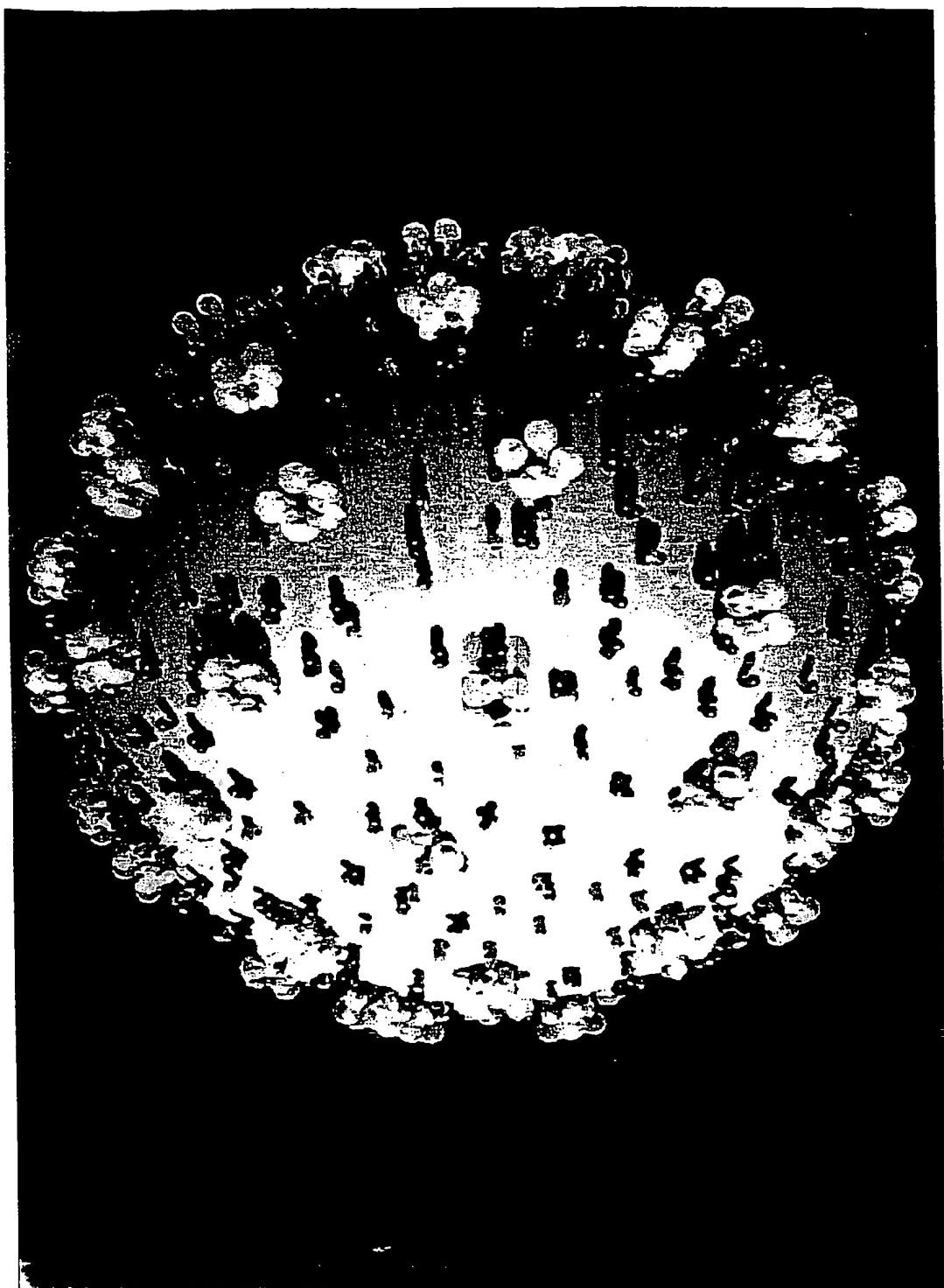


FIGURE 7 (CONT'D) 3

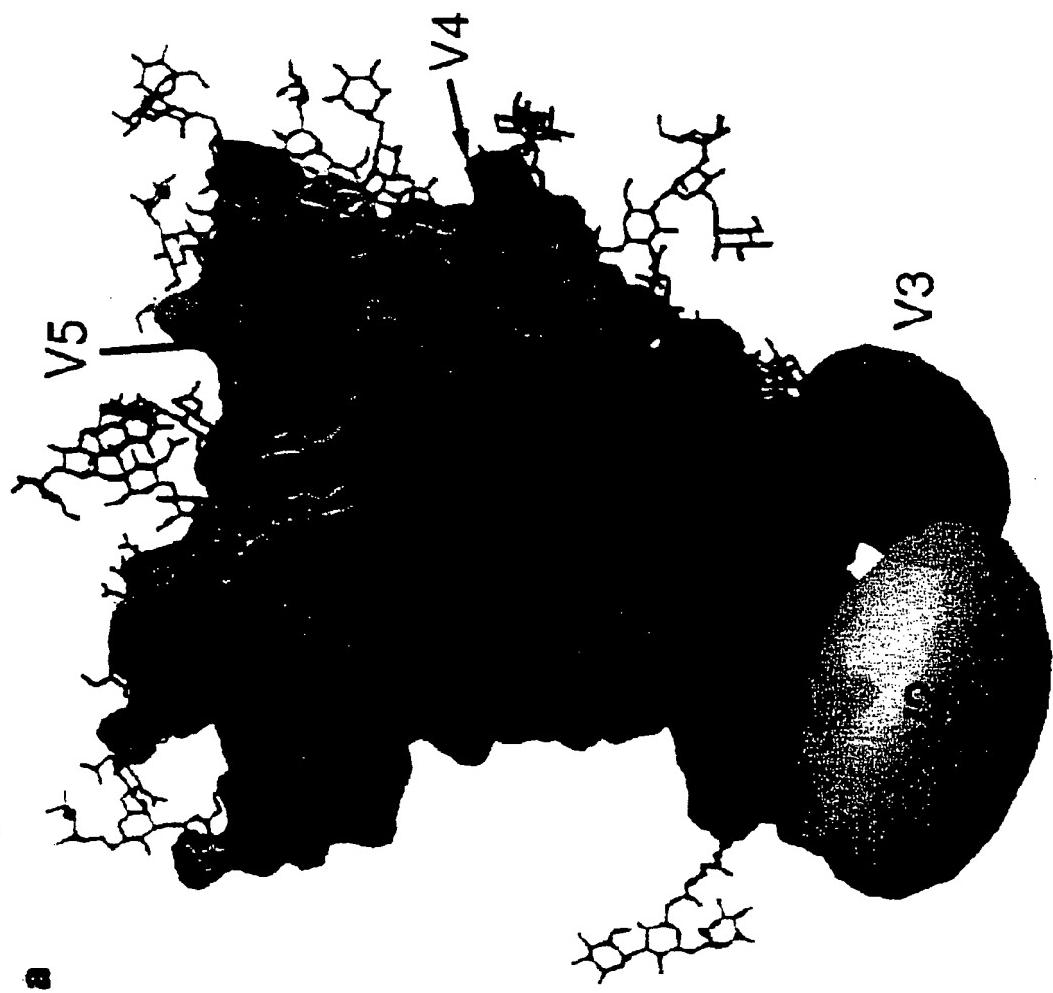


FIGURE 7 (CONT'D) 4

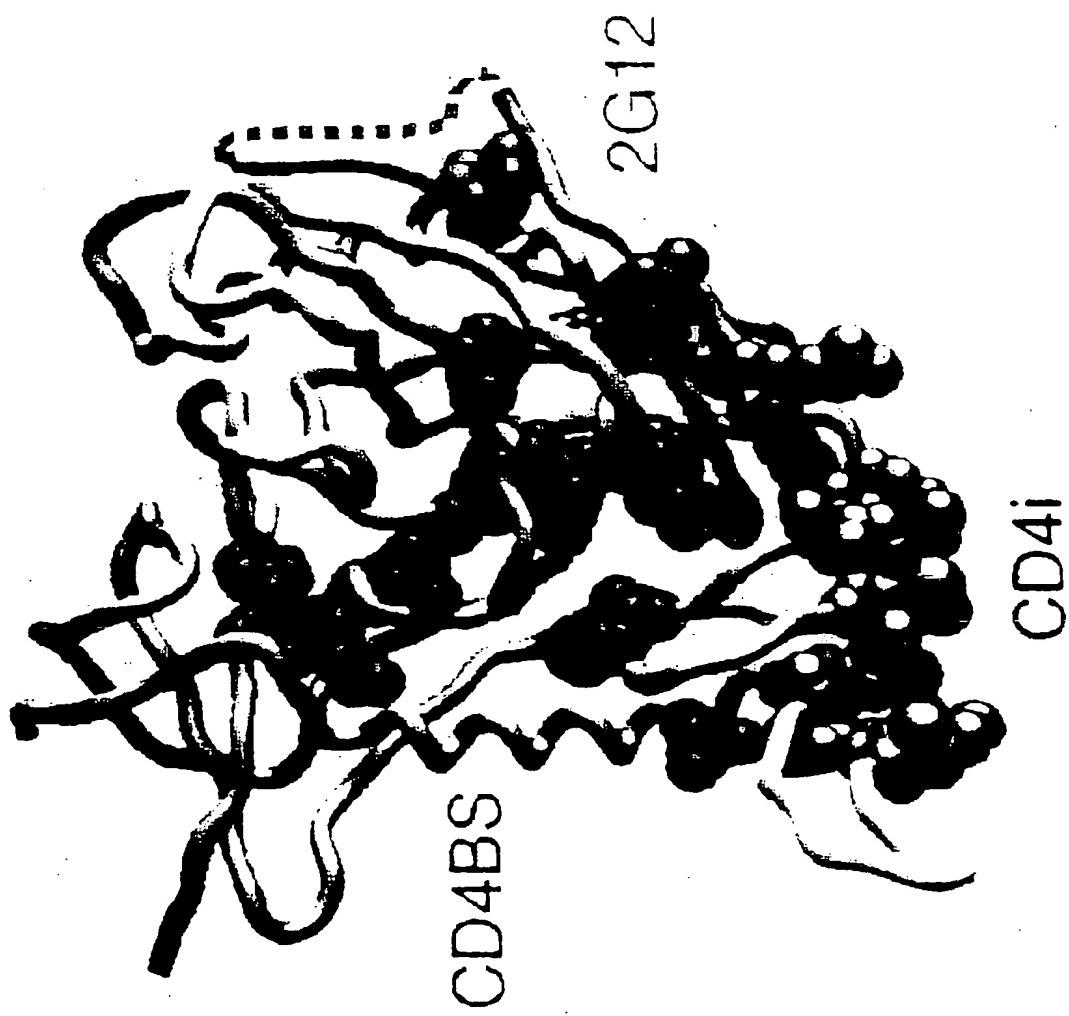


FIGURE 7 (CONT'D) 5

Antibodies to Linear Determinants of the HIV gp120 V3 Loop are Type Specific and Poorly Neutralize HIV Primary Isolates

- HIV-1 grown in T cell lines (TCLA viruses) tend to have basic V3 loops and utilize CXCR4 as a coreceptor.
- In the early 1990s, Carl Hanson at the California Health Dept and Tom Matthews at Duke began to perform neutralizing assays against HIV primary isolates grown only in PBMc. Antibodies to TCLA V3 loops did not neutralize HIV-1 primary isolates.

FIGURE 7 (CONT'D)

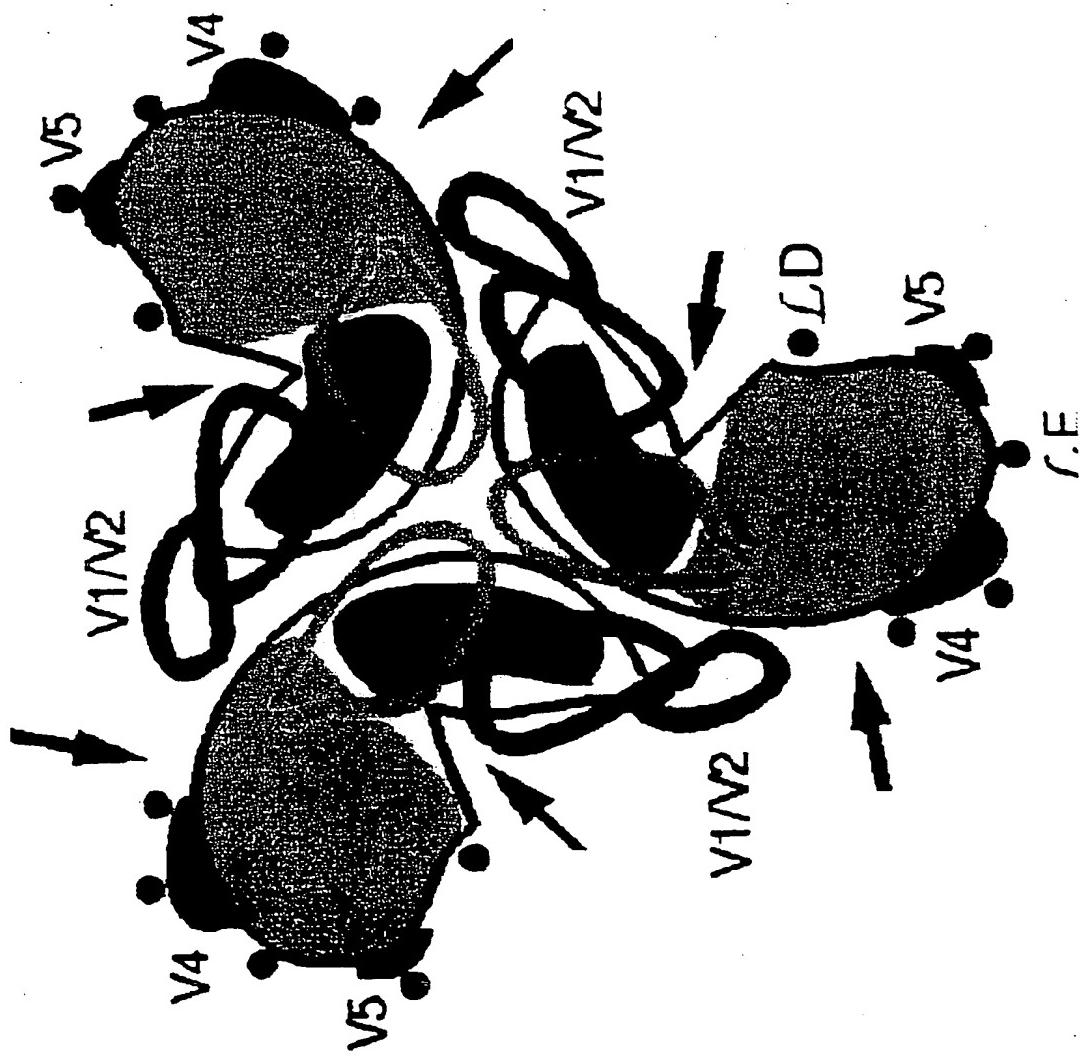


FIGURE 7 (CONT'D) 7

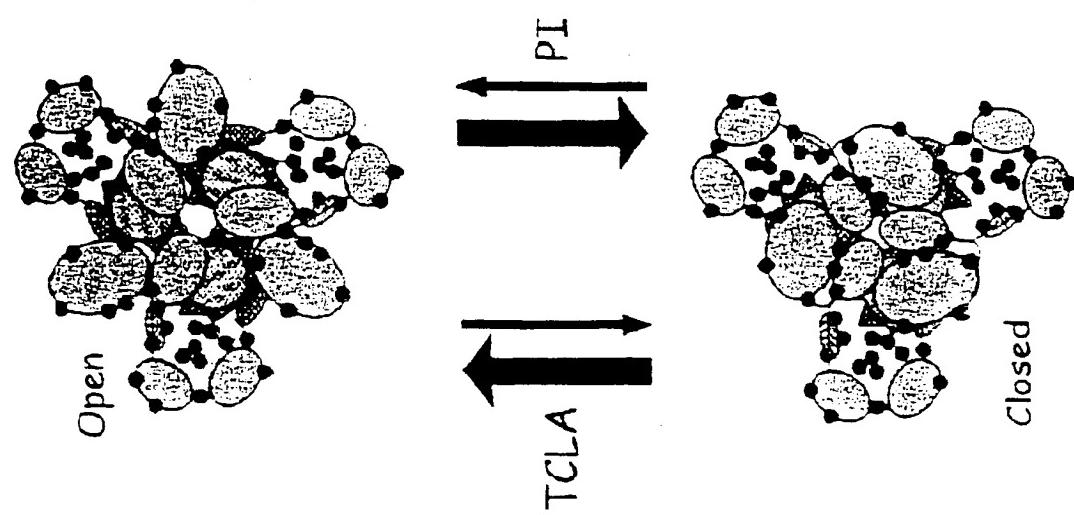


FIGURE 7 (CONT'D) *

Anti-V3 Antibodies Neutralize A Subset of HIV Primary Isolates

- Hioe, C.E., Zolla-Pazner, S. et al. Neutralization of HIV-1 Primary Isolates by Polyclonal and Monoclonal Human Antibodies, Int. Immunol. 9: 1281, 1997.
- Krachmarov, C.P., Pinter, A. et al. V3-specific Polyclonal Antibodies Affinity Purified from Sera of Infected Humans Effectively Neutralize Primary Isolates of HIV-1. AIDS Res. Human Retrovirol. 17: 1737, 2001.

FIGURE 7 (CONT'D) 9

Effect of HIV Envelope gp120 V3 Antibodies

- Neutralize most laboratory adapted HIV
- Neutralize a subset of HIV primary isolates
- Neutralization of HIV to date has been extremely type specific

FIGURE 7 (CONT'D) /D

Vol. 74, No. 1

JOURNAL OF VIROLOGY, Jan. 2000, p. 254-263
0022-538X/00/\$04.00+0
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Induction of Antibodies in Guinea Pigs and Rhesus Monkeys
against the Human Immunodeficiency Virus Type 1 Envelope:
Neutralization of Nonpathogenic and Pathogenic Primary
Isolate Simian/Human Immunodeficiency Virus Strains

HUA-XIN LIAO,^{1,2*} BILIAN ETEMAD-MOGHADAM,³ DAVID C. MONTEFIORI,^{2,4} YING SUN,³
JOSEPH SODROSKI,³ RICHARD M. SCEARCE,^{1,2} ROBERT W. DOMS,⁵ JAMES R. THOMASCH,^{1,2}
SUZANNE ROBINSON,⁶ NORMAN L. LETVIN,⁶ AND BARTON F. HAYNES^{1,2*}

FIGURE 7 (CONT'D) //

Vol. 75, No. 9

JOURNAL OF VIROLOGY, May 2001, p. 4165-4175
0122-538X/01/0404165-11
DOI: 10.1128/JVI.75.9.4165-4175.2001
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Vaccine-Elicited V3 Loop-Specific Antibodies in Rhesus Monkeys and
Control of a Simian-Human Immunodeficiency Virus Expressing
a Primary Patient Human Immunodeficiency
Virus Type 1 Isolate Envelope

NORMAN L. LETVIN,^{1,*} SUZANNE ROBINSON,¹ DANIELA ROHNE,¹ MICHAEL K. AXTHELM,²
JOHN W. FANTON,² MIROSŁAWA BILSKA,³ THOMAS J. PALKER,⁴ HUA-XIN LIAO,³
BARTON F. HAYNES,¹ AND DAVID C. MONTEFIORI¹

FIGURE 7 (CONT'D) 12

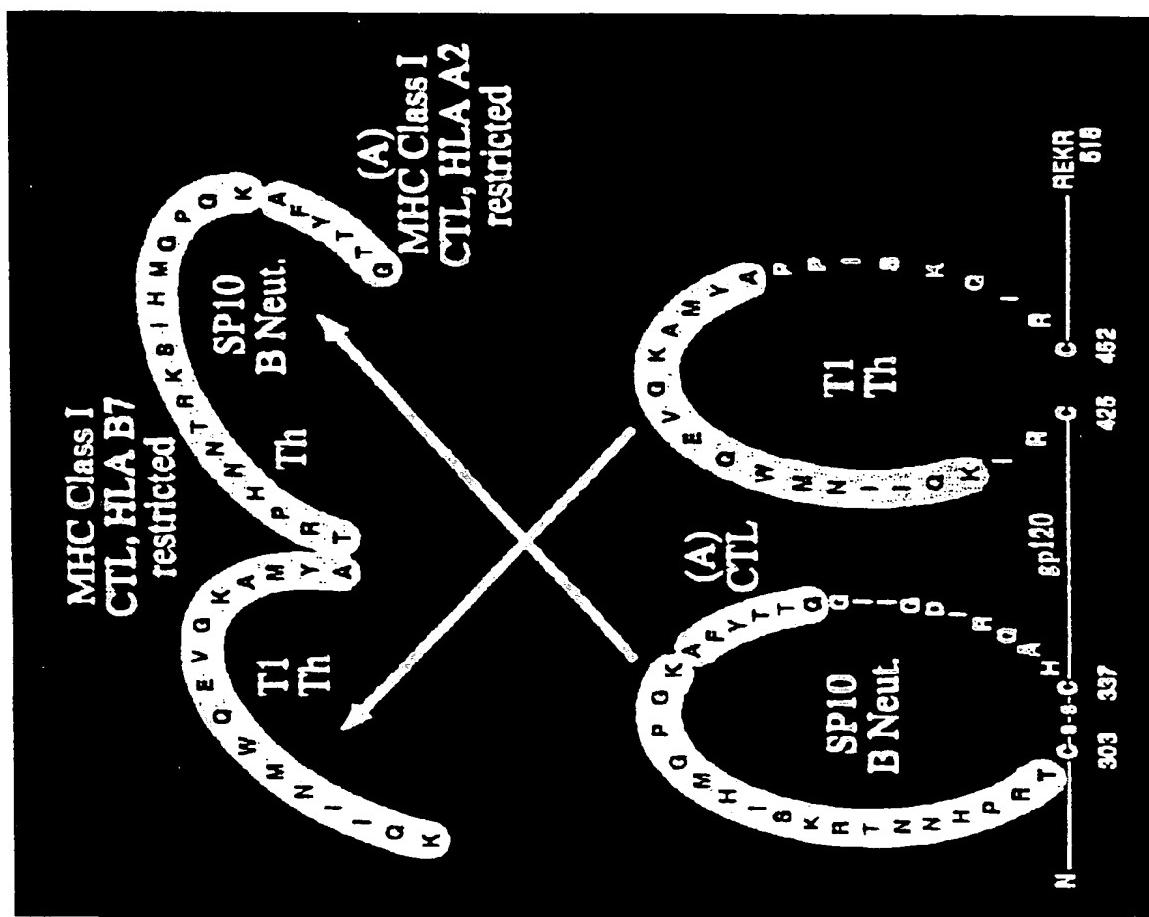


FIGURE 7 (CONT'D) /3

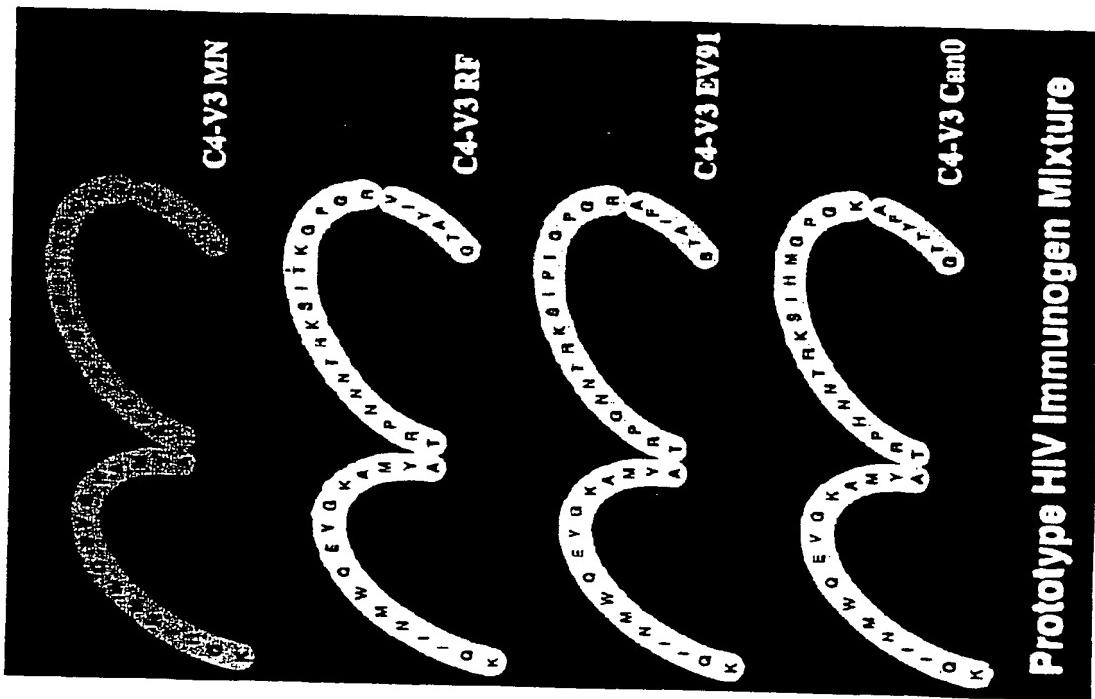


FIGURE 7 (CONT'D) 14

C4-V3 HIV gp120 Peptides

MN	KQIINMMWQEVGKAMYA	TRPNYNKRKRIHTGPGCR	AFYTTTK
B7 epitope	KQIINMMWQEVGKAMYA	TRPNNNTRKSTIKGPGR	VIYATG
RF	KQIINMMWQEVGKAMYA	TRPGNNNTRKSIPIGPGCR	AFIATS
EV91	KQIINMMWQEVGKAMYA	TRPHNNNTRKSIHMGPGK	AFYTTTG
C'ANO			



CanO

EV91

RF

MN

FIGURE 7 (CONT'D) 15

Ability of C4-V3 Peptides to Induce Anti-HIV Neutralizing Antibodies in Rhesus Monkeys to TCLA HIV Isolates After Two or Three Immunizations

Rhesus No.	Immunogen	HIV _{MN}	HIV _{RF}	Neutralization Titer
26252	Polyvalent	>5,000	>6,400	993
26424	C4-V3 _{MN}	[>5,000]	<20	<20
26716	C4-V3 _{RF}	<20	[>6,400]	<20
26906	C4-V3 _{EY91}	[442]	<20	<20
27094	C4-V3 _{ConA}	[4,197]	<20	<20

Vii. et al., J. Virol. 73:746-750, 1999.

FIGURE 7 (CONT'D) /4

6870 CLADE B V3 SEQUENCES = 1514 UNIQUE
FORMS
432 CLADE C V3 SEQUENCES = 176 UNIQUE FORMS

HYPOTHESIS:

SEQUENCE VARIATION FOUND AMONG HIV
PRIMARY ISOLATES NEED NOT REFLECT
THE DIVERSITY OF HIV SEROTYPES, AND
ANTIBODIES MAY CROSS-REACT WITH
GROUPS OF SIMILAR VIRUSES.

FIGURE 7 (CONT'D) 17

METHODS

WE ORGANIZED SHORT ANTIGENIC DOMAINS BY PROTEIN SIMILARITY SCORES USING MAXIMUM-LINKAGE CLUSTERING.

THIS ENABLES VISUALIZATION OF CLUSTERING PATTERNS AS A DENDRITOGRAM, AND THE SPLITTING PATTERN IN THE DENDRITOGRAM CAN BE USED TO DEFINE CLUSTERS OF RELATED SEQUENCES.

KORBER, B.T., ET AL., J. VIROL., 68:6730-6744, 1994.

FIGURE 7 (CONT'D) 18

METHODS

- METHOD ALLOWS THE USE OF SEVERAL DIFFERENT AMINO ACID SCORING SCHEMES.

•AA SUBSTITUTION MATRIX (HENIKOFF AND HENIKOFF, PROTEIN STRUCTURE FUNCTION AND GENETICS, 17:49-61, 1993) WAS DESIGNED TO GIVE AA SUBSTITUTIONS WELL TOLERATED IN CONSERVED PROTEIN STRUCTURAL ELEMENTS A HIGH SCORE, AND THOSE THAT WERE NOT, A LOW SCORE.

FIGURE 7 (CCNT'D) /9

METHODS

- BASED ON THESE CLUSTERING PATTERNS, WE SELECTED A VARIANT THAT WAS MOST REPRESENTATIVE OF EACH GROUP.
- WE EXCLUDED VERY RARE, HIGHLY DIVERGENT SEQUENCES, AND FAVORED SEQUENCES FOUND IN MANY DIFFERENT INDIVIDUALS.
- THIS METHOD ALLOWED FOR MOST OF THE UNIQUE V3 SEQUENCE TO BE WITHIN ONE OR TWO AA FROM AT LEAST ONE OF THE PEPTIDES IN THE COCKTAIL.

FIGURE 7 (CONT'D) 2^o

METHODS

- CLUSTERED 1514 CLADE B V3
SEQUENCES INTO 30 GROUPS.
- CLUSTERED 176 CLADE C V3
SEQUENCES INTO 25 GROUPS.

FIGURE 7 (CONT'D) 2/

Sequences of 30 HIV Clade B C4-V3 Peptides

Peptide Name	Peptide Sequence	Group
C4-V3-23.38	KQI INNMWQVVGKAMYA - RPIKIERKERPIPLGLGKAFAFYTTKG	A
C4-V3-11.85	KQI INNMWQVVGKAMYA - RPNNNTRKSININGPGRFAFYTTG	A
C4-V3-34.29	KQI INNMWQVVGKAMYA - RPNNNTRKSIQIGPGRFAFYTTG	A
C4-V3-1.481	KQI INNMWQVVGKAMYA - RPNNNTRKSIIHGPGRFAFYTTG	A
C4-V3-33.323	KQI INNMWQVVGKAMYA - RPNNNTRKSININGPGRFAFYTTG	A
C4-V3-51.23	KQI INNMWQVVGKAMYA - RPSNNTRRSIIMGLGRAFYTTG	B
C4-V3-36.29	KQI INNMWQVVGKAMYA - RPNNNTRKGKIHIGPGRFFATG	B
C4-V3-57.20	KQI INNMWQVVGKAMYA - RPNRHTGKSIIRMGGLGRRAWHTTR	B
C4-V3-35.29	KQI INNMWQVVGKAMYA - RPNNNTRKRISLGPGRVVYTTG	B
C4-V3-46.26	KQI INNMWQVVGKAMYA - RPDNTIKORLITHIGPGRPEFTT	B
C4-V3-69.18	KQI INNMWQVVCKAMYA - RPNNNTRKSIRIGPGRAVYATT	C
C4-V3-72.18	KQI INNMWQVVGKAMYA - RPNNNTRKGKINICPGRFAFYATG	C
C4-V3-70.18	KQI INNMWQVVGKAMYA - RPNNNTRKRIRIGHIGPGRFAFYATG	C
C4-V3-62.19	KQI INNMWQVVGKAMYA - RPNNNTRKSIIHGPGRFAFYATE	C
C4-V3-74.17	KQI INNMWQVVGKAMYA - RPNNNTRKRMTLGPCKVFEYTTG	C
C4-V3-82.15	KQI INNMWQVVGKAMYA - RPNNNTRRSIPIGPGRFAFYTTG	D
C4-V3-13.1	KQI INNMWQVVGKAMYA - RPNNNTRRSIHLGMGRALYATG	D
C4-V3-89.14	KQI INNMWQVVGKAMYA - RPSINKRRTHIHGPGRFAFYAT	D
C4-V3-85.15	KQI INNMWQVVGKAMYA - RPNNNTRKSIIAPGRFAFYTTG	D
C4-V3-122.9	KQI INNMWQVVGKAMYA - RPENNETKIRIURGYGRSEYTVR	D
C4-V3-170.6	KQI INNMWQVVGKAMYA - RPNNNTRRSVRIGPGAMERTG	E
C4-V3-146.8	KQI INNMWQVVGKAMYA - RPGNNTRRISIGPGRFAVATK	E
C4-V3-163.7	KQI INNMWQVVGKAMYA - RLYNYRRKGTHIGPGRALYATG	E
C4-V3-125.9	KQI INNMWQVVGKAMYA - RPNNNTRRISMGPGRVLYTTC	E
C4-V3-162.7	KQI INNMWQVVGKAMYA - REGNNTRGSIHLHPGRKFYYSR	E
C4-V3-396.2	KQI INNMWQVVGKAMYA - RPNNNTRRNTHIGLGRRFYAT	F
C4-V3-144.8	KQI INNMWQVVGKAMYA - RPDNNIVRK1PICPGSSFYTT	F
C4-V3-365.2	KQI INNMWQVVGKAMYA - RFSNNTRKGTHLGPRAIYATE	F
C4-V3-513.2	KQI INNMWQVVGKAMYA - RFSNNTRKGTHMGPGKAIYTD	F
C4-V3-1448.1	KQI INNMWQVVGKAMYA - REGNNTRRGIPIGPGRFAEFTG	F

FIGURE 7 (CONT'D) 22

HPLC Profile of Purified HIV Clade B
C4-V3 Peptide 46.26

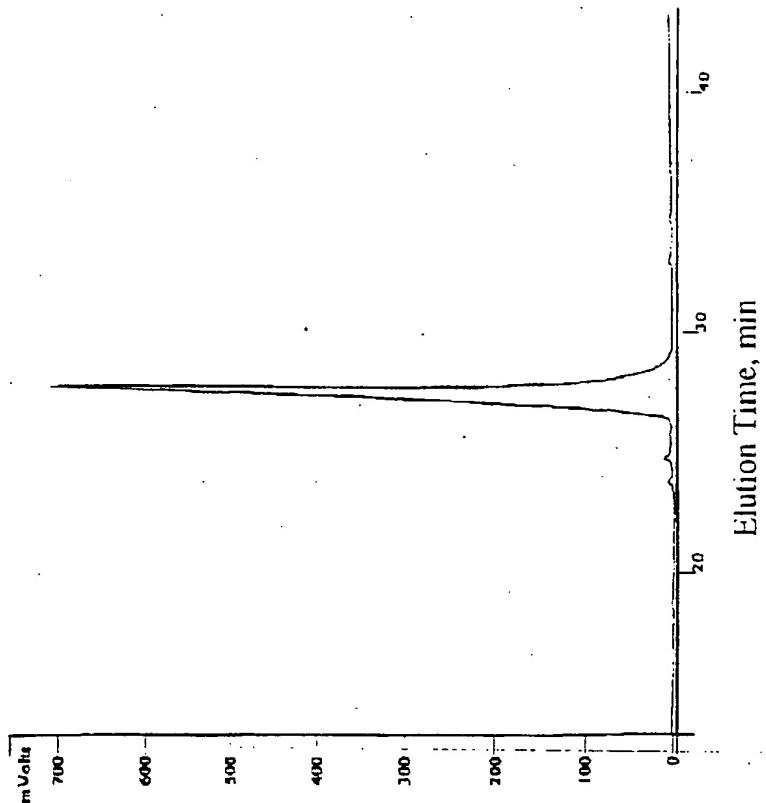


FIGURE 7 (CONT'D) 2.3

**Mass Spectroscopy of Profile of Purified
HIV Clade B C4-V3 Peptide 46.26**

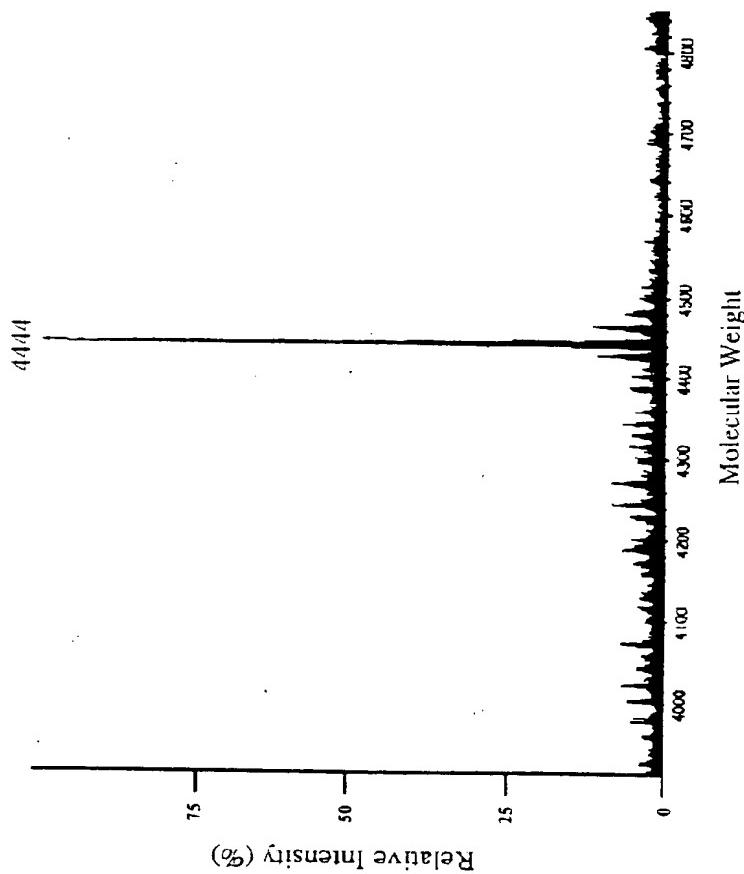


FIGURE 7 (CONT'D) 24

Sequences of 30 HIV Clade B C4- V3 Peptides

Pptide Name	Pptide Sequence	Group
C4-V3-23.38	KQI INNMWQVVGKAMYA- RPNIKIERKIRIPLGLGKAFYTTK	A
C4-V3-11.85	KQI INNMWQVVGKAMYA- RPNNNTRKSININGPGRAYFTTG	A
C4-V3-34.29	KQI INNMWQVVGKAMYA- RPNNNTRKSIIQGPGRAYFTTG	A
C4-V3-1.481	KQI INNMWQVVGKAMYA- RPNNNTRKSIIQGPGRAYFTTG	A
C4-V3-3.323	KQI INNMWQVVGKAMYA- RPNNNTRKSINMGPGRAYFTTG	A
C4-V3-51.23	KQI INNMWQVVGKAMYA- RPSNNTRRSIHMGLGRAFYTTG	B
C4-V3-36.29	KQI INNMWQVVGKAMYA- RPDNNTRKGIHIGPGRFFATG	B
C4-V3-57.20	KQI INNMWQVVGKAMYA- RPNRHTGKSIIRMGLGRAWHTTR	B
C4-V3-35.29	KQI INNMWQVVGKAMYA- RPNNNTRKRISLGPGRVYYIIG	B
C4-V3-16.26	KQI INNMWQVVGKAMYA- RDNTIKORLIHIGPGRPEYT	B
C4-V3-69.18	KQI INNMWQVVGKAMYA- RPNNNTRKSIRIGPGRAYATD	C
C4-V3-72.18	KQI INNMWQVVGKAMYA- RPNNNTRKGRKINIGPGRAYATG	C
C4-V3-70.18	KQI INNMWQVVGKAMYA- RPNNNTRKIRIGHIGPGRAYATG	C
C4-V3-62.19	KQI INNMWQVVGKAMYA- RPNNNTRKSIHIGPGRAYATE	C
C4-V3-74.17	KQI INNMWQVVGKAMYA- RENNTRKRMTLGPKVFTTGG	D
C4-V3-82.15	KQI INNMWQVVGKAMYA- RPNNNTRRSIPIGPGRAYFTTG	D
C4-V3-113.1	KQI INNMWQVVGKAMYA- RPNNNTRRSIHLGMGRALYATG	D
C4-V3-89.14	KQI INNMWQVVGKAMYA- RPSINKRRIHIGPGRAYAT	D
C4-V3-85.15	KQI INNMWQVVGKAMYA- RPNNNTRKSIHAPGRAYFTTG	D
C4-V3-122.9	KQI INNMWQVVGKAMYA- RPNEYNETKIRIIRGYGRSPYTVR	D
C4-V3-170.6	KQI INNMWQVVGKAMYA- RENNTRRSVRIGPGAMFRTG	E
C4-V3-146.8	KQI INNMWQVVGKAMYA- RPGNNTRRISIGPRAFVATK	E
C4-V3-163.7	KQI INNMWQVVGKAMYA- RLNNYRRKGIIHGPRAIYATG	E
C4-V3-125.9	KQI INNMWQVVGKAMYA- RPNNNTRRISMGPGRVLYTTG	E
C4-V3-162.7	KQI INNMWQVVGKAMYA- RPGNNTRGSIHLHPGRKFYYSR	E
C4-V3-396.2	KQI INNMWQVVGKAMYA- RPDNNTRNIHIGLGRRFYAT	F
C4-V3-144.8	KQI INNMWQVVGKAMYA- RPDNNTRVKIPTGPGRSSFTT	F
C4-V3-365.2	KQI INNMWQVVGKAMYA- RPSNNTRKG1HLGPGRALYALE	F
C4-V3-513.2	KQI INNMWQVVGKAMYA- RPSNNTRKG1HMGPGRKAIYTTD	F
C4-V3-1448.1	KQI INNMWQVVGKAMYA- RPGNTTRGIPGPGRAYFTTG	F

FIGURE 7 (CONT'D) 25

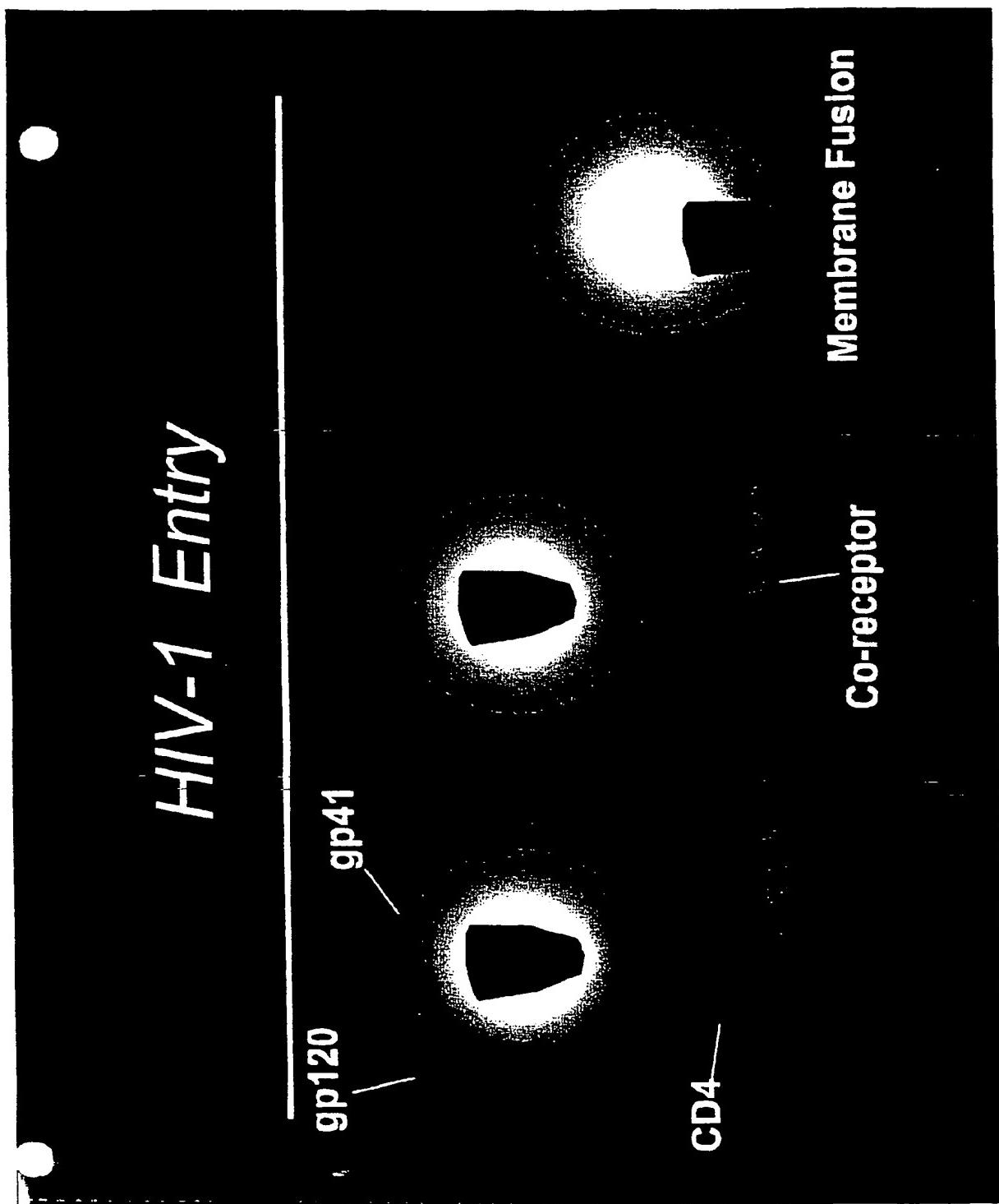


FIGURE 7 (CONT'D) 26

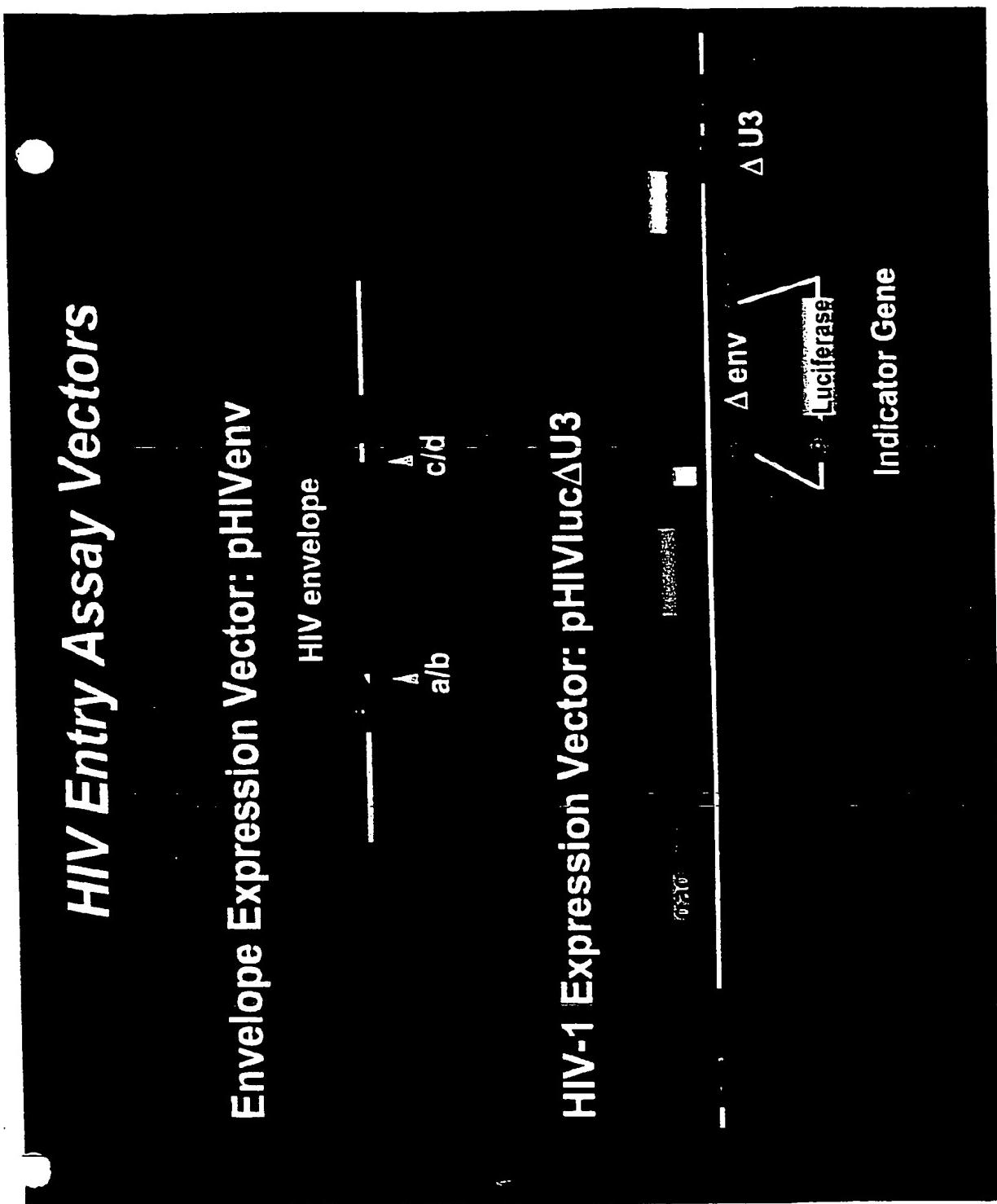
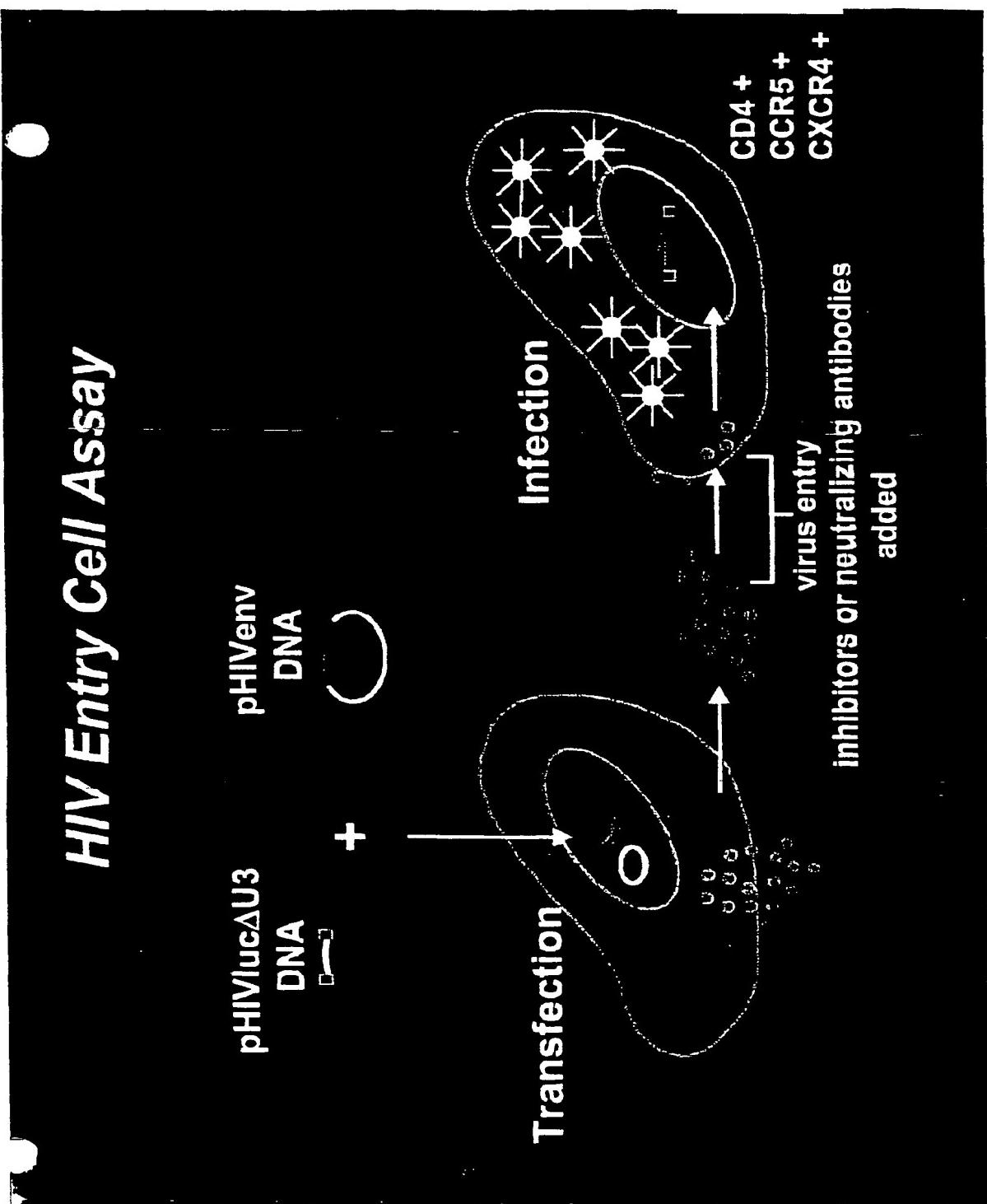


FIGURE 7 (CONT'D) 27



Sequences Of HIV Isolates Used To Screen Clade B V3 Peptide Immunogens

Tropism	Virus	Clade	V3 loop sequence
Dual	Dual A	B	CTRPNNNTRKSIHGPFFAYATGDIIGDIRQAH C
Dual	Dual B	B	CTRPNNNTRKSVTLGPGRWYTTGGIVGDIRXAHC
Dual	Dual C	B	CTRPNNNTRKSVHMGWRRTFFFTEKIGDVRKAHC
Dual	Dual D	AG	CSRPNNNTRXSVRIGPGQTFYATGDXIGDIRQAH C
Dual	Dual E	B	CTRPNNNTSKSHMGA GRAWHATRKIIGDIKKAH X
X4	X4 A	B	CTRHNNSKRTRIGPGRSYYTTKXIXGPIGQAH C
X4	X4 B	B	CTRPNNTRKGIYIGPGRKVYTEXKITGDMKKAYC
X4	X4 C	B	CTRPNNHTQRRIAGPGRSFYATDRIXGDLKQAH C
X4	X4 D	B	CIRPNNSAKAIRIGPGRAVIAKRXIGNRQAH C
R5	1196	ND	CTRPNNNTRKSIHGPGRAYXTGEIIGDIRQAH C
R5	BAL	ND	CTRPNNNTRKSIHGPGRAYXTGEIIGDIRQAH C
R5	JRFL	ND	CTRPNNNTRKSIHGPGRAFYTTGEIIGDIRQAH C
R5	SF162	ND	CTRPNNNTRKSIHGPGRAYATGDIIGDIRQAH C
R5	TORNO	ND	CTRPNNNTRRSIHGPGRAYATGDIIGDIRQAH C
R5	PAVO	ND	CTRPNNNTRKSIHGPGRAYATGDIIGDIRQAH C
R5	6101 (P15)	ND	CTRPNNNTRKSIHMGPGAAFYARGEVIGDIRQAH C
R5	692	ND	CTRPGNNTTRKSIHGPGRAYATGDIIGDIRQAH C
R5	5768 (P27)	ND	CTRPNNNTRKSIHMGPKVFYTTGEIIGDIRQAH C
R5	1168	ND	CERPNNNTIKSILGPGRAWHATGQIIGDIRKAFC
R5	515	ND	CTRPNNNTRKSIHAGKALYTGEIIGDIRQAH C

FIGURE 7 (CONT'D) 2 Y

FIGURE 7 (CONT'D) 29

Rank Order of V3 Sequences Based on Ability to Neutralize 19 Clade B HIV Primary Isolates

396.2	R P N N N T R N I H I G L G R R F Y A T -	0
170.6	R P N N N T R R S V R I G P G G A M F R T G	0
23.38	R P I K I E R K R I P L G L G K A F Y T T K	0
113.1	R P N N N T R R S I H I L G M G R A L Y A T G	0
51.23	R P S N N T R R S I H I M G L G R A F Y T T G	0
57.20	R P N R H T G K S I R M G L G R A W H T T R	0
35.29	R P N N N T R K R I S L G P G R V Y Y T T G	0
122.9	R P N Y N E T K R I R H G Y G R S F V T V R	0
144.8	R P D N N T V R K I P I G P G S S F Y T T -	1
365.2	R P S N N T R K G I H L G P G R A I Y A T E	1
125.9	R P N N N T R R I S M G P G R V I Y T T G	1
162.7	R P G N N T R Q S I H L I P G R K F Y Y S R	1
46.26	R P D N T I K Q R I I H I G P G R P F Y T T -	2
3.323	R P N N N T R K S I N M G P G R A F Y T T G	2
82.15	R P N N N T R S I P I G P G R A F Y T T G	2
1448.1	R P G N T T R R G I P G P G R A F F T T G	3
69.18	R P N N N T R K S I R I G P G R A V Y A T D	3
72.18	R P N N N T R K G I N I G P G R A F Y A T G	3
70.18	R P N N N T R K R I R I G H I G P G R A F Y A T G	3
89.14	R P S I N K R H I H I G P G R A F Y A T	3
163.7	R L Y N Y R R K G I H I G P G R A F Y A T	3
85.15	R P N N N T R K S I H I A P G R A F Y T T G	3
513.2	R P S N N T R K G I H M G P G K A I Y T T D	3
1.481	R P N N N T R K S I H I G P G R A F Y T T G	4
11.85	R P N N N T R K S I N I G P G R A F Y T T G	4
74.17	R P N N N T R K R M T L G P G K V F Y T T G	5
36.29	R P N N N T R K G I H I G P G R T F F A T G	6
34.29	R P N N N T R K S I Q I G G P G R A F Y T T G	8
62.19	R P N N N T R K S I H I G P G R A F Y A T E	16

FIGURE 7 (CONT'D) 30

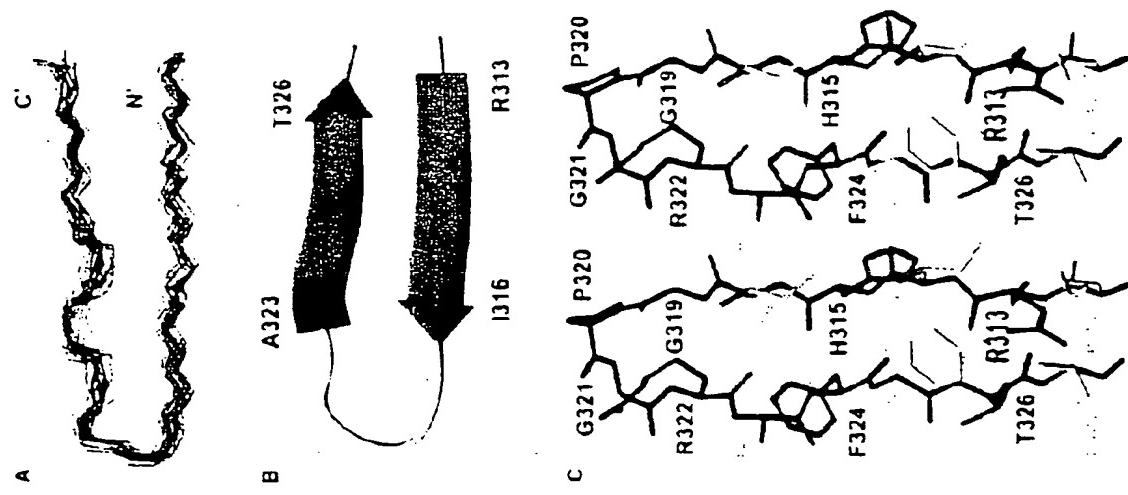


FIGURE 7 (CONT'D) ^{3/}

Rank Order of V3 Sequences Based on Ability to Neutralize 19 Clade B HIV Primary Isolates

Peptide Neutralized	V3 Sequence	Isolates
396.2	R P N N N T R R N	L R AT-
170.6	R P N N N T R S V R	G M F R I G
23.38	R P I K I E R R P L	TTK 0
113.1	R P N N N T R R S L	M L ATG 0
122.9	R P N Y N E T R H R Y	S V T V R 0
57.20	R P N R H T G S R M L	W H T T R 0
35.29	R P N N N T R S L	V Y T T G 0
51.23	R P S N N T R R S M L	T T G 0
144.8	R P D N N T V R K P	S S TT 0
365.2	R P S N N T R G L	I A T E 1
125.9	R P N N N T R R S M	V L T T G 1
162.7	R P G N N T R Q S L I I	K Y S R 1
46.26	R P D N T I K Q R I	P T T 2
3.323	R P N N N T R S N M	T T G 2
82.15	R P N N N T R R S P G	T T G 3
1448.1	R P G N T T R R G P	F T T G 3
69.18	R P N N N T R S R	V A T D 3
72.18	R P N N N T R C N	F A T D 3
70.18	R P N N N T R R G H I	F A T G 3
89.14	R P S I N K R R H	A T G 3
163.7	R L Y N Y R R G	I A T G 3
85.15	R P N N N T R S A	T T G 3
513.2	R P S N N T R G M	K I T T D 4
1.481	R P N N N T R S	T T G 4
11.85	R P N N N T R S	T T G 5
74.17	R P N N N T R M T L	K V T T G 5
36.29	R P N N N T R G	T F A T G 6
34.29	R P N N N T R S Q	T T G 8
62.19	R P N N N T R S	A T E 16

447 MAB region

FIGURE 7 (CONT'D) ³²

Ability of HIV ADA gp120 and gp140 Envelope Proteins To Induce Antibodies That Neutralize HIV Primary Isolates

Guinea Pig No.	Immunogen	MN [*]	Neutralizing Titer Against HIV-1 isolates					
			SHIV 89.6 ^{**}	ADA	SS1196	JRF1	SF162	BAL
Reciprocal Dilution of neutralizing antibody titer								
518A	ADA gp120	8,900	43	<20	183	<20	<20	29
531	ADA gp120	451	<20	<20	<20	<20	<20	202
532	ADA gp120	5,700	<20	<20	200	<20	<20	76
533	ADA gp120	1,863	30	<20	146	<20	<20	197
	GMT	2,555*	6	<20	48	<20	<20	2†
								126
519	ADA gp140	14,614	<20	NT	259	<20	<20	34
520	ADA gp140	21,492	48	58	237	<20	24	224
521	ADA gp140	15,788	85	<20	441	<20	<20	84
522	ADA gp140	18,667	43	61	237	<20	46	34
	GMT	17,378*	20	<20	280	<20	6	39†
								203

^{**}MT-2 Assay; all other HIV-1 isolates tested in the M7-Luciferase Assay. Values are the reciprocal serum at which infectivity reduced by 80% relative to no sample (50% for ADA).

*p = .029; † = p=.017

FIGURE 7 (CONT'D) 33

**Ability of HIV ADA gp120 and gp140 Envelope Proteins
To Induce Antibodies That Inhibit Syncytium Formation
From Without**

Guinea Pig No.	Immunogen	Reciprocal Dilution Of GP Serum To Inhibit $\geq 90\%$ of Syncytium Formation Induced by AT-2-Inactivated HIV-1 ADA
518A	ADA gp120	2,430
531	ADA gp120	270
532	ADA gp120	810
533	ADA gp120	810
<hr/>		
519	ADA gp140	2,430
520	ADA gp140	>2,430
521	ADA gp140	2,430
522	ADA gp140	2,430

FIGURE 7 (CONT'D) 34

Ability of C4-V3 62.19 and 34.29 HIV ENV gp120 Subunit Immunogen to Neutralize HIV Primary Isolates

Immunogen	GP#	Neutralizing Titer Against HIV Primary Isolates					
		BAL	BX08	QH0692	6101	BG1168	SF162
C4-V3 62.19	609	293	172	286	89	50	119
	610	211	220	103	35	<20	21
	611	186	86	125	38	54	124
	612	129	49	338	50	30	390
C4-V3 34.29	605	88	254	148	20	26	413
	606	41	156	144	<20	26	>540
	607	30	71	26	23	34	>540
	608	46	59	163	31	26	240
Positive Control LEH03 HIV+ Human Serum		2401	1846	978	1618	217	>5400

FIGURE 7 (CONT'D) 35

**Ability of C4-V3 36.29 and 1.481 HIV ENV gp120
Subunit Immunogen to Neutralize HIV Primary Isolates**

Immunogen	GP#	BAL	Neutralizing Titer Against HIV Primary Isolates					S1196
			BX08	QH0692	6101	BC1168	SF162	
C4-V3 36.29	617	233	196	72	<20	<20	158	245
	618	183	108	90	23	30	>540	130
	619	121	95	62	<20	<20	48	56
	+ FA	620	166	214	40	<20	<20	65
C4-V3 1.481	597	98	167	72	39	<20	63	66
	598	167	230	90	44	<20	21	99
	599	65	195	62	<20	<20	244	68
	+ FA	600	99	119	40	20	<20	178
C4-V3 57.20 + FA	464	<20	22	<20	<20	<20	<20	<20
Positive Control LEH03 HIV+ Human Serum		3,665	1,514	624	NT	404	3,535	3,129

FIGURE 7 (CONT'D) 36

**Comparison of C4-V3 62.19 HIV ENV Immunogen
Serum Neutralizing Antibody Titers When Formulated
in Freunds Adjuvant Versus RC529 + Mutant Cholera Toxin**

Immunogen	GP#	Neutralizing Titer Against HIV Primary Isolates					
		BAL	BX08	QH0692	6101	BG1168	SF162
C4-V3 62.19 +	609	293	172	286	89	50	119
	610	211	220	103	35	<20	21
	611	186	86	125	38	54	124
	612	129	49	338	50	30	390
C4-V3 62.19 +	634	446	386	249	35	37	172
	635	119	44	127	21	<20	516
	636	319	179	221	<20	22	31
	637	100	213	116	21	<20	438
+							
m CT							
Positive Control LEH03 HIV+ Human Serum		2401	1846	978	1618	217	>5400

FIGURE 7 (CONT'D) 37

HIV gp120 V3 Sequences of HIV Bal, HIV 89.6 and HIV ADA Compared to C4 -V3 Peptides

HIV Bal V3 CTRPNNNTRKSIHIGPGRAFYTTGEIIGDIRIQAHC
HIV ADA V3 CTRPNNNTRKSIHIGPGRAFYTTGEIIGDIR-QAHC

V3 1.481 TRPNNNTRKSIHIGPGRAFYTTG
V3 62.19 TRPNNNTRKSIHIGPGRAFYATE
V3 36.29 TRPNNNTRKGIHIGPGRTFFATG
V3 34.29 TRPNNNTRKSIQIGPGRAFYTTG

V3 57.20 TRPNRHTGKSIRMGGLGLRAWHTTR

V3 MN TRPNYNKRKRIHIGPGRAFYTTK

FIGURE 7 (CONT'D) 3⁸

HIV gp120 V3 Sequences of HIV Bal, HIV 89.6 and HIV ADA Compared to C4-V3 Peptides

HIV Bal V3	CTRPNNTTRKSIHIGPGRAFYTTGELIGDIRIQAHC
HIV ADA V3	CTRPNNTTRKSIHIGPGRAFYTTGELIGDIR-QAHC
HIV 89.6 V3	CTRPNNTTRRLSIGPGRAFYARRNIIGDIR-QAHC
V3 1.481	TRPNNNTTRKSIHIGPGRAFYTTG
V3 62.19	TRPNNNTTRKSIHIGPGRAFYATE
V3 36.29	TRPNNNTTRKGIIHIGPGRTFFATG
V3 34.29	TRPNNNTTRKSIIQIGPGRAFYTTG
V3 57.20	TRPNRHTGKSIRMMGLGLRAWHTTR
V3 MN	TRPNYNKRKRIHIGPGRAFYTTK

FIGURE 7 (CONT'D) ³⁹

Comparison of HIV gp120 Bal, gp120 89.6, and V3 Bal to Induce Antibodies that Neutralize HIV Primary Isolates

Guinea Pig No.	Immunogen	Neutralizing Titer Against HIV Primary Isolates					
		Bal	BX08	BG1168	6101	SF162	SS1196
21	C4-V3 Bal	404	>540	25	110	324	>540
22	C4-V3 Bal	69	487	28	99	39	183
							86
573	gp120 Bal	>540	>540	<20	<20	>540	295
574	gp120 Bal	>540	>540	<20	<20	>540	380
575	gp120 Bal	>540	479	<20	<20	352	70
							21
507	gp 120 89.6	<20	102	<20	<20	>540	<20
508	gp 120 89.6	<20	189	<20	<20	>540	47
509	gp 120 89.6	<20	<20	<20	<20	386	<20
							22

Assays performed in the M7-Luciferase assay and are titers at which 50% neutralization occurs.

FIGURE 7 (CONT'D) ^{4D}

Ability of HIV BAL and HIV 89.6 gp120 Envelopes to Induce Antibodies that Neutralize HIV Primary Isolate

Guinea Pig No.	Immunogen	HIV Isolate					
		Bal	89.6	SF162	JRCSF	BL01	BR07
573	HIV Bal gp120*	82	90	80	88	29	81
574	HIV Bal gp120*	100	67	82	77	0	75
578	HIV Bal gp120*	99	68	85	64	32	30
568	HIV 89.6 gp120#	0	98	81	0	0	72
507	HIV 89.6 gp120†	0	99	84	47	0	99
508	HIV 89.6 gp120†	32	97	81	73	36	98
509	HIV 89.6 gp120†	0	96	73	48	50	90
							42

* RiBi - CWS Adjuvant

RiBi - MPL-SE + MCT Adjuvant

† CFA / IFA - Adjuvant

Assays is single round infection assay of intracellular p24 determined by flow cytometry.

All sera tested at 1:10 dilution, and data are percent reduction of p24.

FIGURE 7 (CONT'D) 4/

HIV gip120 V3 Sequences of HIV Bal, HIV 89.6 and HIV
ADA Compared to C4-V3 Peptides

HIV Bal V3	CTRPNNTTRKSIHIGPGRAFYTTGEIIGDIRIQAHC
HIV ADA V3	CTRPNNTTRKSIHIGPGRAFYTTGEIIGDIR.QAHC
V3 1.481	TRPNNNTTRKSIHIGPGRAFYTTG
V3 62.19	TRPNNNTTRKSIHIGPGRAFYATE
V3 36.29	TRPNNNTTRKGIHIGPGRTFFATG
V3 34.29	TRPNNNTTRKSIQIGPGRAFYTTG
V3 57.20	TRPNRHTGKSIRMGLGLRAWHTTR

FIGURE 7 (CONT'D) 42

HIV gp120 V3 Sequences of HIV Bal, HIV 89.6 and HIV
ADA Compared to C4-V3 Peptides

HIV Bal V3 CTRPNNNTRKSIHIGPGRAYTTGEIIGDIRIQAHC
HIV ADA V3 CTRPNNNTRKSIHIGPGRAYTTGEIIGDIR.QAHC
HIV 89.6 V3 CTRPNNNTRRRLSIGPGRAYARRNIIGDIR.QAHC

V3 1.481 TRPNNNTRKSIHIGPGRAYTTG
V3 62.19 TRPNNNTRKSIHIGPGRAYATE
V3 36.29 TRPNNNTRKGIHIGPGRFFATG
V3 34.29 TRPNNNTRKSIQIGPGRAYTTG

V3 57.20 TRPNRHTGKSIRMGLGLRAWHTTR

FIGURE 7 (CONT'D) ⁴³

**HIV gp120 V3 Sequences of HIV Bal, HIV 89.6 and HIV
ADA Compared to C4-V3 Peptides**

HIV Bal V3 C T R P N N N T R K S I H I G P G R A F Y T T G E I I G D I R I Q A H C
HIV ADA V3 C T R P N N N T R K S I H I G P G R A F Y T T G E I I G D I R - Q A H C

V3 1.481 T R P N N N T R K S I H I G P G R A F Y T T G

FIGURE 7 (CONT'D) #4

**HIV gp120 V3 Sequences of HIV Bal, HIV 89.6 and HIV
ADA Compared to C4-V3 Peptides**

HIV Bal V3 CTRPNNTRKSIHIGPGRAFYTTGEIIGDIRIQAHC
V3 1.481 TRPNNTTRKSIHIGPGRAFYTTG

FIGURE 7 (CONT'D) 45

HIV gp120 V3 Sequences of HIV Bal, HIV 89.6 and HIV ADA Compared to C4-V3 Peptides

HIV Bal V3 CTRPNNNTRKSIHIGPGRAYTTGEIIGDIRIQAH
HIV ADA V3 CTRPNNNTRKSIHIGPGRAYTTGEIIGDIR.QAH
HIV 89.6 V3 CTRPNNNTRRRLSIGPGRAYARRNIIGDIR.QAH

V3 1.481	TRPNNNTRKSIHIGPGRAYTTG
V3 62.19	TRPNNNTRKSIHIGPGRAYATE
V3 36.29	TRPNNNTRKGIIHIGPGRFFATG
V3 34.29	TRPNNNTRKSIQIGPGRAYTTG
V3 57.20	TRPNRHTGKSIRMGGLRAWHTTR
V3 MN	TRPNYNKRKRIHIGPGRAYTTK
V3 MN +	KR
	TTKNIIG

Sharon, M. et al. Structure 11: 223,
2003

FIGURE 7 (CONT'D) 44

HIV gp120 V3 Sequences of HIV Bal, HIV 89.6 and HIV ADA Compared to C4-V3 Peptides

HIV Bal V3	C TRP N N N T R	S					
HIV ADA V3	C TRP N N N T R	S					
HIV 89.6 V3	C TRP N N N T R R L S						
V3 1.481	T R P N N N T R	S					
V3 62.19	T R P N N N T R	S					
V3 36.29	T R P N N N T R	G					
V3 34.29	T R P N N N T R	S Q					
V3 57.20	T R P N R H T G	S R M	L	L R A W H T T R			
V3 MN	T R P N Y N K R				T T K		
V3 MN +	K R				T T K N I I G		
Sharon, M. et al. Structure 11: 223, 2003							

FIGURE 7 (CONT'D) 47

HIV gp120 V3 Sequences of HIV Bal, HIV 89.6 and HIV ADA Compared to C4-V3 Peptides

HIV Bal V3	CTR PNN NTR K S	R F T T G E I I G D I R Q A H C
HIV ADA V3	CTR PNN NTR K S	R F T T G E I I G D I R - Q A H C
HIV 89.6 V3	CTR PNN NTR R R L S	R F A R R N I I G D I R - Q A H C
V3 1.481	T R P N N N T R K S	R F T T G
V3 62.19	T R P N N N T R K S	R F A T E
V3 36.29	T R P N N N T R K G	R T F F A T G
V3 34.29	T R P N N N T R K S I Q	R F Y T T G
V3 57.20	T R P N R H T G K S I R M L L R A W H T T R	
V3 MN	T R P N Y N K R K I	R F T T K
V3 MN +	K R K I	R F T T K N I I G
Sharon, M. et al.	Structure 11: 223,	
2003		

V3 Amino Acids Important For CCR5 Binding
 Wang, W. Et al. Proc. Natl. Acad. Sci., USA 96: 4558,
 1999.

FIGURE 7 (CONT'D) 48

Rank Order of V3 Sequences Based on Ability to Neutralize 19 Clade B HIV Primary Isolates

Peptide Neutralized	V3 Sequence	Isolates
396.2	R P N N N T R R N I H I G L G R R F Y A T .	0
170.6	R P N N N T R R S V R I G P G G A M F R T G	0
23.38	R P I K I E R K I P I L G L G K A F Y T K	0
113.1	R P N N N T R R S I H L G M M G R A L Y A T G	0
51.23	R P S N N T R R S I H M G L G R A F Y T T G	0
57.20	R P N R H T G K S I R M G L G R A W H T T R	0
35.29	R P N N N T R K R I S L G P G R N V Y T T G	0
122.9	R P N Y N E T H R K I R H G Y G R S F V T V R	0
144.8	R P D N N T V R K I P I G P G S S F Y T T .	1
365.2	R P S N N T R K G I H L G P G R A I Y A T E	1
125.9	R P N N N T R R I S M G P G R V L Y T T G	1
102.7	R P Q N N T R G S I H L H P G R K F Y Y S R	1
46.26	R P D N T I K Q R I I H I G P G R P F Y T T .	2
3.323	R P N N N T R K S I N M G P G R A F Y T T G	2
82.15	R P N N N T R S I P I G P G R A F Y T T G	2
1448.1	R P G N T T R R G I P I G P G R A F F T T G	2
69.18	R P N N N T R K S I R G P G R A V Y A T D	3
72.18	R P N N N T R K G I N I G P G R A F Y A T G	3
70.18	R P N N N T R K R I G H I G P G R A F Y A T G	3
89.14	R P S I N K R R H I H I G P G R A F Y A T .	3
163.7	R L Y N Y R R K G I H I G P G R A I Y A I G	3
85.15	R P N N N T R K S I H I A P G R A F Y T T G	3
513.2	R P S N N T R K G I H M G P G K A I Y T D	4
1481	R P N N N T R K S I H I G P G R A F Y T T G	4
1185	R P N N N T R K S I N I G P G R A F Y T T G	5
74.17	R P N N N T R K R M T L G P G K V F Y T T G	5
36.29	R P N N N T R K G I H I G P G R T F F A T G	6
34.29	R P N N N T R K S I Q I G P G R A F Y T T G	8
82.19	R P N N N T R K S I H I G P G R A F Y A T E	16

447 MAB region KRIHIGPGRAFY

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